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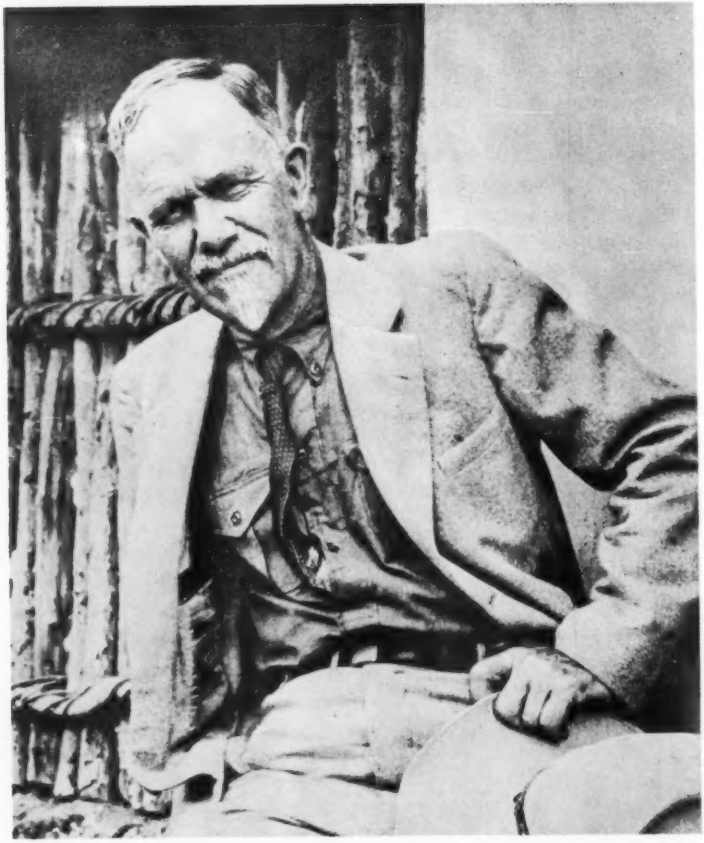

Charles B. Davenport

CHARLES BENEDICT DAVENPORT, 1866–1944

CHARLES BENEDICT DAVENPORT was one of the pioneers in genetics and among the first to apply the new science to man. During the first decade of the twentieth century he published nine papers on human heredity and eugenics, and his contributions in this field numbered more than 100 at the time of his death. Other contributions in genetics included papers on poultry, canaries, and sheep. In all, his scientific writings numbered about 450.

DR. DAVENPORT'S breadth of interest was remarkable. Other than genetics and eugenics, his publications include many articles on growth, anthropology, ecology, and statistical methods. In addition to research, a considerable portion of his time was devoted to administrative duties, to which he brought a never-failing vigor and enthusiasm. In 1898 he became the third director of the BIOLOGICAL LABORATORY of the BROOKLYN INSTITUTE OF ARTS AND SCIENCES, at Cold Spring Harbor, a position which he held until 1923. At that time the Laboratory was incorporated as the LONG ISLAND BIOLOGICAL ASSOCIATION, and a full-time director was appointed, DR. DAVENPORT becoming secretary of the Association.

One of the outstanding scientific events in America occurred in the early days of this century, when, as a result of an endowment by ANDREW CARNEGIE, the CARNEGIE INSTITUTION OF WASHINGTON came into existence. In 1902, at the request of the trustees, various biologists prepared plans for a biological department of the Institution. DAVENPORT'S plans were accepted, and he was asked to organize the department, to be known as the STATION FOR EXPERIMENTAL EVOLUTION. The new station was opened in 1904, at Cold Spring Harbor, on land adjoining the BIOLOGICAL LABORATORY. DR. DAVENPORT was its first director, and held this post until he retired in 1934.

The station, later known as the DEPARTMENT OF GENETICS, grew rapidly in equipment, facilities, and staff, until today it is one of the outstanding genetics research laboratories of the world, with a personnel of nearly 70.

Not only did DR. DAVENPORT have enthusiasm and vision in connection with his administrative ability, but he had the happy faculty of inspiring others with his fervor. In 1906 he interested MR. and MRS. E. H. HARRIMAN in the work of the Station. In 1910, following the death of MR. HARRIMAN, he received from MRS. HARRIMAN funds for a building, the necessary land adjacent to the Station, and finally an endowment, for a EUGENICS RECORD OFFICE for the preservation of records of human family histories and for the study of human heredity.

The EUGENICS RECORD OFFICE trained many field workers and published many papers on human genetics and eugenics. In 1918 it was incorporated into the CARNEGIE INSTITUTION OF WASHINGTON and was combined with the STATION FOR EXPERIMENTAL EVOLUTION as the DEPARTMENT OF GENETICS, with DAVENPORT in charge.

DR. DAVENPORT'S influence was strongly felt in the establishment of the EUGENICS RESEARCH ASSOCIATION, under the auspices of which some im-

portant studies in eugenics were made, including those on birth rates, the biological effects of war, and the inheritance and social implications of mental differences. His influence and guidance also were instrumental in the founding of the EUGENICAL NEWS, which, beginning in 1916, published monthly articles and comments on human heredity and eugenics.

In reviewing DR. DAVENPORT'S life, it is impossible to forget the profound personal influence he had upon research workers in the field of genetics. The proximity of the BIOLOGICAL LABORATORY, the STATION FOR EXPERIMENTAL EVOLUTION, and the EUGENICS RECORD OFFICE resulted in the bringing together of many investigators and large numbers of students, especially during the summer sessions. DR. DAVENPORT was an inspiration to all of these. To the mature investigator he was a friendly, critical colleague, a wholly unselfish source of suggestions and information. To the student and the beginning worker he was a wise, patient, and inspiring counsellor, never too busy to give his time and advice, never too involved in work to listen to the hopes and plans of eager students. His death brought not only a scientific loss, but a very personal void to many geneticists both here and abroad.

DR. DAVENPORT had traveled to Europe eight times, had been once to Australia, and had journeyed far and wide over the North American continent in the pursuit of his scientific activities. He was a member of many foreign scientific societies and held offices in most of the important American biological societies. His presidencies include those of the AMERICAN SOCIETY OF ZOOLOGISTS, the GALTON SOCIETY, the EUGENICS RESEARCH ASSOCIATION, and the INTERNATIONAL FEDERATION OF EUGENIC ORGANIZATIONS. He received the Gold Medal of the NATIONAL INSTITUTE OF SOCIAL SCIENCES in 1923. He was a member of the NATIONAL ACADEMY OF SCIENCES, and a fellow of the AMERICAN ACADEMY OF ARTS AND SCIENCES. During World War I he was a major in charge of anthropology, attached to the Surgeon General's office.

CHARLES DAVENPORT was born June 1, 1866, at Stamford, Conn., the son of a school teacher. He received the B.S. from the BROOKLYN POLYTECHNIC INSTITUTE in 1886, the A.B. from HARVARD UNIVERSITY in 1889, and the Ph.D. from HARVARD in 1892. He remained there as an instructor until 1899, moving to the UNIVERSITY OF CHICAGO as assistant professor and later associate professor until 1904, when he assumed the directorship of the STATION FOR EXPERIMENTAL EVOLUTION. In 1894 he married GERTRUDE CROTTY of Burlington, Kansas. They had two daughters and a son.

DR. DAVENPORT continued his research activities after his retirement in 1934 until in the early days of 1944 he contracted a cold which developed rapidly into pneumonia. He died on February 18, 1944.

LAURENCE H. SNYDER

A STUDY IN GENE ACTION USING DIFFERENT DOSAGES AND ALLELES OF VESTIGIAL IN DROSOPHILA MELANOGASTER[1]

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Received July 25, 1945

THE method of gene action in *Drosophila melanogaster* has been studied from several different approaches. The earliest attempts involved determination of the effect of the external environment on the manifestation of mutant genes. MORGAN (1915) and BRAUN (1938) have reported mutants which were affected by moisture content of the media. KRAFKA (1920) determined that the number of facets in Bar-eye imagoes was inversely proportional to the temperature of larval development. Other investigators analyzed this temperature effect, leading to the discovery that the temperature is effective during a particular interval of larval development. Subsequent investigations have shown that temperature can alter the phenotype in a number of Drosophila mutants. STANLEY (1931, 1935) and HARNLY (1930, 1936) have reported that a larval temperature greater than 29°C causes a significant increase in wing length in homozygous vestigial individuals. A high developmental temperature will cause fewer dorsocentral bristles to occur in the mutant Dichaete (PLUNKETT 1926) and will also affect the number of bristles in scute flies (CHILD 1935).

A more positive method of analysis of gene action has been the study of the effects of different dosages of the mutant genes upon the adult phenotype. STERN (1929) studied the bobbed mutant of *D. melanogaster*, which produces bristles of reduced size. He found that the addition of bobbed genes was associated with an increase in the length of the bristles, causing them to approach the wild phenotype. In general, the same conditions have been found to apply to the wing-venation associated with the gene *ci* in the fourth chromosome (STERN 1943). SCHULTZ (1935) reported that an increase in the number of shaven genes in *D. melanogaster* resulted in a phenotype approaching the normal bristles. MULLER (1932) used the method of overlaps to increase the dosage of genes and found that added genes for eosin or apricot produced an eye color approaching that of the normal eye. However, the addition of a gene for ebony, on the third chromosome, produced a darker body color—that is, the tendency here was away from normal.

The study of the effect of prolonged larval life upon the adult phenotype has led to some inferences as to the nature of the action of the genes studied. Two methods have been utilized to prolong larval life. One involves raising the larvae on a "starvation" diet. The other makes use of known mutant genes which act to increase the duration of larval life. Feeding flies upon a low food

[1] The data reported upon in this paper have been taken from the dissertation submitted to the Graduate School, UNIVERSITY OF MINNESOTA, in September 1942, in partial fulfillment of requirements for the degree of Doctor of Philosophy.

level in order to prolong larval life, BODENSTEIN (1939) observed that the number of facets in Bar was often significantly increased when larval life was thus prolonged. CHILD (1939) observed that "starvation" resulted in an increase in the wing length of homozygous vestigial individuals. BRAUN (1942) reported that the amount of destruction of wing area in vg^{No} and in ct^{n} is increased by partial starvation. Investigators who have used genes to prolong larval life have obtained somewhat contrasting results. MARGOLIS (1935), using the prolongation effect associated with vestigial, reported that the facet number in Bar-eye was significantly decreased by the association of Bar with homozygous vestigial. DUNN and COYNE (1935) used dominant Minutes to prolong larval life and found that a significant decrease in the size of the eyes occurred in Lobe^{2} individuals which were heterozygous for Minute. BRYSON (1940) found that prolongation with Minutes resulted in an increase in the penetrance of a number of dominant and recessive gene mutations affecting the eyes and wings. GREEN and OLIVER (1940) reported that prolongation of the larval life caused by Minutes or by a duplication of the third chromosome resulted in scalloping of the wings of heterozygous vestigial individuals, which would normally resemble the wild type. GARDNER (1942) made a study of the effects of several mutants upon *vg* dominigene action and reported that many have an effect either as enhancers or as inhibitors of the gene action.

Although these references in no sense give a complete report of the numerous studies on gene action, they indicate the various methods of approach. The data to be presented in this report are based upon studies of the effects of temperature and prolongation of larval life upon the action of the vestigial-wing gene in *D. melanogaster*. The effects of variable increases in the duration of larval life as associated with three different Minute genes were studied in heterozygous vestigial individuals and also in duplication individuals possessing either one or two genes for vestigial with the other two or one, respectively, being the normal allele. The effects on a number of vestigial alleles are also reported.

MATERIALS AND METHODS

The mutant vestigial-wing used in the study is caused by a recessive autosomal gene (*vg*) located on the second chromosome of *Drosophila melanogaster*. From cytological analyses of known deficiencies, BRIDGES (see MORGAN, BRIDGES, STURTEVANT, and SCHULTZ 1938) concluded that the position of *vg* is within region 49 of the salivary chromosome map. The homozygous *vg* phenotype is characterized by a reduction of the wings to tiny stubs, a reduction in the size of the halteres, and an alteration the direction of the scutellar bristles. In the heterozygous condition *vg* is completely suppressed by its normal allele, and the phenotype is wild type. Alleles of *vg* which were used in the studies include the following: vestigial-nick (vg^{ni}), which when homozygous produces no visible alteration in the wings; vestigial-nick of Green (vg^{nG}), which when homozygous produces only occasional nicks in the wings; and vestigial-no wing (vg^{nw}) and vestigial-Notch-2 (vg^{No2}), each of which when homozygous

causes a decrease in the wing and haltere size greater than that observed in homozygous vestigial.

For the study of the effects of different dosages of *vg* on the wing phenotype, individuals having a duplication of a small portion of the second chromosome, including the locus of *vg*, were selected from among progeny of flies that were heterozygous for two translocations involving the second and fourth chromosomes. These translocations, *T(2, 4)A40* and *T(2, 4)A29*, and the methods used to produce hyperploids are described by PATTERSON, BROWN, and STONE (1940), who graciously sent the cultures to us. In translocation *T(2, 4)A40* the second chromosome is broken at 49F (salivary chromosome map), which is just to the right of *vg*. The right end fragment of the chromosome is attached to chromosome four. In translocation *T(2, 4)A29* the second chromosome is broken at 47A, to the left of the *vg* locus. The right portion of the second chromosome, including the region of *vg*, is attached to the fourth chromosome.

In order to get recessive vestigial into a duplication stock which could be used in the study of dosage effects, *T(2, 4) A29 vg⁺ey⁺/Cy* flies were mated to *vg ey*, and the non-*Cy* females were mated back to *vg ey*. One male among more than 5000 offspring from this backcross mating was *vg ey⁺* (vestigial-wing, non-eyeless flies) and proved to have the genotype *T(2, 4)A29 vg ey⁺/vg ey*, with the recessive *vg* crossed into the piece of chromosome two transocated to chromosome four. This male was mated to Curly, eyeless females, and a balanced culture *T(2, 4)A29 vg ey⁺/Cy vg⁺ ey* was prepared. This culture is kept in the stocks at the UNIVERSITY OF MINNESOTA.

In order to develop the duplication cultures the following scheme of matings was used (symbols for the fourth chromosome genes *ey* and *ey⁺* are omitted):

P_1 *Cy/T(2, 4)A40 al vg⁺ sp* × *Cy/T(2, 4)A29 al⁺ vg sp⁺*
F_1 ♂ *T(2, 4)A40 al vg⁺ sp/T(2, 4)A29 al⁺ vg sp⁺* × ♀ *Cy/al Bl sp*
F_2 ♂ progeny with the phenotype *al Bl* were selected and mated to *Cy/Gla*.
F_3 ♂ ♀ *Cy/Bl⁺* were selected and bred giving a constant breeding duplication stock.

(The symbols used represent the following second chromosome genes: *al*, aristaless; *Bl*, Bristle; *sp*, speck; *Cy*, Curly; *Gla*, Glazed; and *vg*, vestigial.) All the genes in each fragment of a translocation or in each complete chromosome are underscored with a continuous line.

In the outlined crosses, the duplication individuals segregate out in the F_2 generation. All F_2 individuals showing the *al Bl* phenotype have the portion of *T(2, 4)A40*, which is marked in the left arm with *al* and which is broken just beyond the *vg* locus and carries *vg⁺*. They receive the other portion of chromosome two from *T(2, 4)A29*. This fragment includes the loci from *vg* through to the right end of the chromosome and is marked by *vg* and *sp⁺*. The mating of the F_2 *al Bl* ♂ to *Cy/Gla* was made in order to establish a culture carrying the duplication balanced to Curly. These cultures have three vestigial loci, one *vg* and one *vg⁺* in the fragments of the two translocations and one *vg⁺* on the unbroken second chromosome marked with Curly.

Prolongation of the larval life was accomplished by the introduction of each of the three dominant Minutes, $M(2)l^2$, $M(3)w$, and $M(1)n$, into the genome. These genes cause a reduction in the size of the bristles, prolongation of the larval life, and lethality when homozygous. BREHME (1939, 1941) made careful measurements of the extent of prolongation of the larval life associated with each of these Minutes and found that at a temperature of 25°C, $M(2)l^2$ prolonged the total larval life the least, $M(3)w$ intermediately, and $M(1)n$ the most. However, she found that $M(2)l^2$ delays the third larval instar by approximately 13 hours; $M(1)n$ by 30 hours; and $M(3)w$ by 38 hours.

All matings were carried out in a uniform manner. Ten or twelve pairs of parents were allowed to mate in vials for 48 hours and then were transferred to paper spoons which were covered with the regular banana-agar medium. The females were allowed to oviposit for 10–12 hours, after which time the spoons were removed and replaced by fresh ones. Eggs were collected and distributed in lots of 75–125 to half-pint milk bottles containing about 50 cc of the regular banana-agar medium. (In a few of the last experiments, it was necessary to use a cornmeal-agar medium instead of the banana-agar medium.) Such a procedure eliminated the effects of crowding and insured uniformity of environmental effects. All offspring were allowed to develop in a constant temperature chamber at a temperature of 23 ± 1°C, unless otherwise stated.

EXPERIMENTAL DATA

Effect of Minutes on vg/+

The influence on $vg/+$ individuals of prolongation of larval life by $M(2)l^2$ and $M(3)w$ was tested by mating homozygous *vg* females to the respective Minute males and to wild type males. $M(1)n$, on the X chromosome and lethal in males, was introduced by mating females carrying that gene to *vg* males. On the assumption that *vg* is recessive to its normal allele, all the offspring from these matings are expected to have normal wings. It is clear from the data tabulated in table 1 that the introduction of the Minutes into the $vg/+$ individuals resulted in a modification of the wing phenotype away from normal. This modification is expressed in the form of scalloping (nicking and notching) of the wings. With each Minute, the frequency of scalloping is greater than that observed in the controls. Although only 0.77 per cent of the control ($vg/+$) females had nicked wings, 31.2 per cent of the $vg/+$, $M(2)l^2$ females had scalloped wings. The frequencies of scalloping in $vg/+$, $M(1)n$ and $vg/+$, $M(3)w$ females were 72.44 and 87.6 per cent, respectively. A greater proportion of males than females in each genotype had nicked wings. These results agree in general with those reported by GREEN and OLIVER (1940). However, the data in the present study are reported because they were obtained with a better control of crowding and because they were run concurrently with tests on dosage and on the alleles of vestigial.

It is noteworthy that there seems to be a correlation between the frequency of wing scalloping and the degree of prolongation of the third larval instar associated with the Minutes. The lowest frequency of wing scalloping was ob-

served in *vg*/+, *M*(2)*l*^{2}, in which the third larval instar is prolonged least; the frequency in *vg*/+, *M*(1)*n* was intermediate; and in *vg*/+, *M*(3)*w*, where there is the greatest prolongation of this instar, the frequency of wing scalloping was the highest.

It will be useful to reserve the term "penetrance" to denote simply the expression or non-expression of the mutant gene, regardless of degree; thus it is penetrance that is described above. On the other hand, degree of expression

TABLE 1

Influence of prolongation of larval life on different dosages of vg. Under genotype, + refers to the normal allele of vg; the Minute in each instance is heterozygous; the extra vg locus is present in a small duplication of the second chromosome.

GENOTYPE	SEX	NO. FLIES	PERCENTAGE NORMAL	PERCENTAGE ONE WING SCALLOPED	PERCENTAGE TWO WINGS SCALLOPED
vg/+	♀	130	99.23	0.77	0.00
	♂	108	94.45	5.55	0.00
vg/+, *M*(2)*l*^{2}	♀	266	68.80	26.31	4.89
	♂	255	38.04	41.96	20.00
vg/+, *M*(1)*n*	♀	156	27.56	39.10	33.33
vg/+, *M*(3)*w*	♀	121	12.40	40.49	47.11
	♂	100	1.00	15.00	84.00
vg/+/+	♀	240	100.00	0.00	0.00
	♂	251	99.20	0.80	0.00
vg/+/+, *M*(2)*l*^{2}	♀	348	98.27	1.73	0.00
	♂	414	92.03	6.52	1.45
vg/+/+, *M*(3)*w*	♀	218	83.94	15.14	0.92
	♂	304	80.92	13.49	5.59
vg/*vg*/+	♀	166	96.99	3.01	0.00
	♂	189	84.66	14.81	0.53
vg/*vg*/+, *M*(2)*l*^{2}	♀	109	18.35	25.69	55.59
	♂	139	24.46	23.74	51.80
vg/*vg*/+, *M*(3)*w*	♀	47	2.13	2.13	95.74
	♂	64	1.56	1.56	96.88

can conveniently be measured by comparisons of the percentages of individuals with one wing and with both wings scalloped. Here too there is apparent an association with the prolongation of the third larval instar. Thus of the *vg*/+

M(2)l² females 4.89 per cent had both wings nicked, approximately one-fifth as many as those having nicks in one wing only. In *vg/+*, *M(1)n* females 33 per cent had both wings nicked, only slightly fewer than those having a single wing nicked. In *vg/+*, *M(3)w* females 47.1 per cent had both wings nicked, slightly more than the proportion with one nicked wing. The degree of expression, like the penetrance, was much greater in males than in females. Twenty per cent of the *M(2)l²* males and 84 per cent of the *M(3)w* males had both wings scalloped. No control flies were observed to have both wings scalloped.

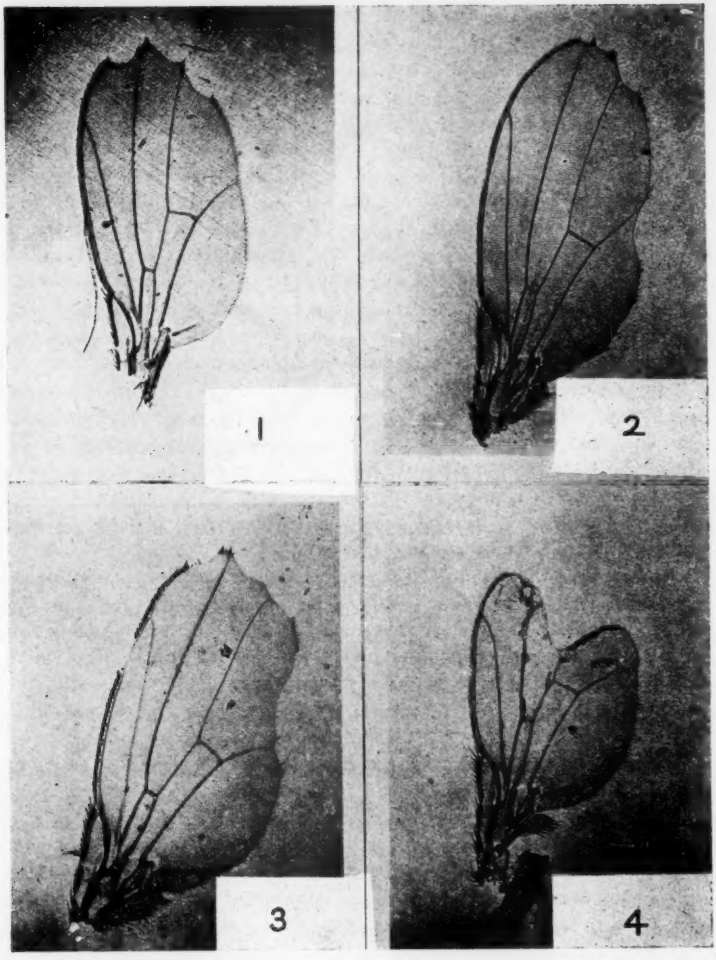
Because of the variability in the degree and region of the wing scalloping, no quantitative measurements of the reduction in wing size were attempted. However, certain trends in the general degree and region of the wing scalloping were easily detected. Among the *vg/+*, *M(2)l²* females, scalloping occurred in the inner margin and only occasionally at the apex of the wing. Among the *vg/+*, *M(2)l²* males, scalloping usually appeared as a notch or nick in the apical region of the wing (plate fig. 1). Scalloping in the *vg/+*, *M(1)n* females occurred either at the apex or in the inner or outer margin. Occasionally both the apex and one or the other of the margins were scalloped simultaneously. This was also found to be true for both females and males of the *vg/+*, *M(3)w* genotype. By far the most extreme manifestations were encountered in the *vg/+*, *M(3)w* males (plate fig. 2).

Effect of Minutes on vg/+/+

The effect of one vestigial gene against two of its normal alleles was studied by the use of the duplication of salivary regions 47A–49F, as described under methods. The duplication complex carried *vg* in that portion of the second chromosome from *T(2, 4)A29* which is attached to chromosome four, and a normal allele (*vg⁺*) in the part of the second from *T(2, 4)A40*. The other second chromosome, carrying *vg⁺*, was derived from the mating of the duplication culture to wild type, *M(2)l²*, or *M(3)w*.

The interactions between one dose of *vg* and two normal alleles under these conditions are shown in table 1. Even in the presence of two normal alleles, one *vg* functioned occasionally to cause wing scalloping in some flies of both sexes when either of the two Minutes was present in the genome. In females *vg/+/+*, *M(3)w*, which have the longer third instar developmental time, the vestigial gene showed a greater penetrance than in *vg/+/+*, *M(2)l²* females. Only 1.73 per cent of the *M(2)l²* females, as compared to 16.06 per cent of those carrying *M(3)w*, had scalloped wings. A slightly greater proportion of males than females in each combination had wings that were scalloped. Nicking or scalloping was observed in none of 240 *vg/+/+* (control) females.

There was a marked decrease in the degree of expression of *vg* in *vg/+/+* as compared with *vg/+*, again using percentage of two-wing scalloping as the measure. None of the *vg/+/+*, *M(2)l²* females and less than two per cent of the males had both wings scalloped. Less than one per cent of the *vg/+/+*, *M(3)w* females and less than six per cent of the males had both wings scalloped. Although prolongation of the third instar of larval development does increase the effectiveness of one vestigial gene in competition with two of its normal



FIGURES 1–4.—All the photographs are magnified ×28. Figure 1.—Wing of *vg*/+, *M*(2)^{P}♂ showing scalloping in the apical region. Figure 2.—Wing of *vg*/+, *M*(3)*w*♂ with scalloping in apex and inner margin. Figure 3.—Wing of *vg*/*vg*/+, *M*(2)^{P}♀ with scalloping in apex and both the inner and outer margins. Figure 4.—Wing of *vg*/*vg*/+, *M*(3)*w*♂ showing effect of scalloping which reduced the size of the wing.

alleles, the effect is less marked than when it is in competition with only one.

With but few exceptions, all cases of scalloping were confined to nicks and slight notches in the apical region of the wing. The exceptions were represented by occasional nicks in the inner and outer margins of the wings of *vg*/+/+, *M*(3)*w* individuals.

Effect of Minutes on vg/vg/+

The Minutes were brought into combination with two dosages of recessive *vg* and one of the normal allele by mating flies of the duplication culture to either *Cy/vg*, *M(2)l²* or *Cy/vg*, *M(3)w/C3X* flies. The frequencies of nicking with the various combinations are included in table 1. Some difficulty was encountered in obtaining individuals of the genotype *vg/vg/+*, *M(3)w*, probably because of the poor fertility of the *Cy/vg*, *M(3)w/C3X* males. As will be pointed out however the results of the action of *M(3)w* on *vg/vg/+* were so striking that the paucity of such individuals can hardly invalidate the data obtained. It is apparent from the data that prolongation of the larval life had a pronounced effect on flies of this genotype. Approximately 3 per cent of the *vg/vg/+* females (controls) and 15.3 per cent of the control males had scalloping of the wings. However, the frequency of scalloping of the Minute, *vg/vg/+* individuals was in each case significantly greater than in the controls. Scalloping of the wings occurred in 81.65 per cent of the *vg/vg/+*, *M(2)l²* females and 97.87 per cent of the *vg/vg/+*, *M(3)w* females. It is interesting to note that the same degree of sexual difference found in the studies of *vg/+* and *vg/+/+* flies did not occur here. In the *vg/vg/+* controls the proportion of males with notched wings was considerably higher than that of the females. However, with *M(2)l²* fewer males than females were affected, and with *M(3)w* almost all of the males and females were scalloped.

The addition of the Minutes to the *vg/vg/+* complex increased not only the penetrance but also the degree of expression of the vestigial genes. With the *vg/vg/+*, *M(2)l²* approximately twice as many flies had both wings involved as had only one wing nicked. In the *vg/vg/+*, *M(3)w* almost all of the flies had both wings scalloped. Finally, both the pentrance and the degree of expression of *vg* in Minute, *vg/vg/+* individuals were clearly more marked than in Minute, *vg/+* flies.

In all cases the prolongation effect on the size of the wing was striking. In the *vg/vg/+*, *M(2)l²* individuals scalloping was observed as extreme notches in the apical region of the wing, with incisions often occurring in the inner and outer margins (fig. 3). The scalloping appeared to be even more extreme than that observed in *vg/+*, *M(3)w* males. In the *vg/vg/+*, *M(3)w* individuals an extreme reduction in the wing size was observed. The wings were incised simultaneously in the apical and inner margins; and occasionally the apex and inner and outer margins were all incised, giving the wing the appearance of the Beadex phenotype. In a number of individuals the wings were reduced to approximately one-half the normal size (fig. 4). In no individual, however, was any reduction in haltere size observed.

It is appropriate at this time to evaluate the data which have been presented. From the analysis of the effects of the Minutes on different quantities of the *vg* gene, a number of conclusions are suggested. In association with the prolongation of the third instar of larval life, the recessive *vg* gene in heterozygous condition manifests itself in the form of scalloping of the adult wings. The frequency and degree of wing scalloping are found to be roughly propor-

tional to the extent of prolongation of the third larval instar of development and also to the number of *vg* genes present in the genome. It should be noted that the action of the Minute gene seems to be related solely to its effect on the duration of a definite interval of larval life and not to a specific modifying action of the Minute itself. This is indicated by the similar effects which have been observed by using other genetic factors to prolong larval life. A duplication of part of the third chromosome [*Dp(3)T(1, 3)O5*], the haplo-four condition, and the sex-linked mutant kurz, all of which prolong larval life, produce scalloping in *vg*/+ individuals (GREEN and OLIVER 1940 and unpublished). Moreover, DUNN and COYNE (1935) and BRYSON (1940) found that Minutes modify the action of certain other dominant and recessive genes.

Under the conditions used in the study—that is, with recessive *vg* carried in a small duplicated piece of the second chromosome—one dosage of *vg* is able to express itself occasionally in males when in combination with two of its normal alleles. The action of one *vg* in competition with two normal alleles (*vg*/+/+) is not as great as it is in competition with only one normal allele (*vg*/+), while two recessive vestigial genes in combination with one normal allele (*vg*/*vg*/+) produce the most extreme effects on the wings. Prolongation of the larval life associated with the Minutes leads with each dosage of *vg* to an increased frequency and degree of wing scalloping as compared to the respective control. This development away from normal is most extreme in those flies which have the extreme prolongation of the third larval instar associated with *M(3)w*.

Effect of Minutes on vestigial alleles

In view of the findings that prolongation with Minutes is associated with increased scalloping of the wings in *vg*/+ individuals, tests were made to determine whether or not comparable effects occurred with some of the alleles of vestigial. The alleles selected for study were: no-wing (vg^{nw}) and Notch 2 (vg^{No2}), each of which when homozygous causes a decrease in the size of the wing and haltere greater than that observed in homozygous *vg;* nick (vg^{ni}) which when homozygous produces no visible alteration in the phenotype; and nick-of-Green (vg^{nG}) which when homozygous produces only occasional nicks in the wings.

vg^{nw} *and* vg^{No2}

Heterozygous vg^{nw} and heterozygous vg^{No2}, two extreme alleles of vestigial, were tested by mating females from stock cultures to $M(2)l^2$, *M(3)w*, and wild type males. Matings were made in the manner previously described, and development took place at a temperature of 23°C.

The results of the matings are given in table 2. The results with heterozygous vg^{No2} closely parallel those with vg^{nw}, and therefore a discussion of the latter will suffice for a description of both. Scalloping was observed in a small proportion of the control individuals (vg^{nw}/+) of both sexes. With the addition of the Minutes, the frequency of scalloping was increased considerably; 87.9 per cent

TABLE 2

Influence of prolongation of larval life on alleles of vg. Under genotype, + refers to the normal allele of vg; the Minute in each instance is heterozygous.

GENOTYPE	SEX	NO. FLIES	PERCENTAGE NORMAL	PERCENTAGE ONE WING SCALLOPED	PERCENTAGE TWO WINGS SCALLOPED
$vg^{nw}/+$	♀	135	97.78	2.22	0.00
	♂	123	95.12	4.06	0.81
$vg^{nw}/+$, $M(2)l^2$	♀	190	12.10	27.37	60.53
	♂	187	7.49	22.99	69.52
$vg^{nw}/+$, $M(3)w$	♀	96	0.00	0.00	100.00
	♂	149	0.67	0.67	98.66
$vg^{Nd}/+$	♀	127	95.27	4.73	0.00
	♂	122	93.44	4.92	1.64
$vg^{Nd}/+$, $M(2)l^2$	♀	130	5.38	15.38	79.23
	♂	131	6.11	14.50	79.39
$vg^{Nd}/+$, $M(3)w$	♀	64	0.00	0.00	100.00
	♂	102	0.98	0.98	98.02
vg^{ni}/vg^{ni}	♀	255	100.00	0.00	0.00
	♂	195	100.00	0.00	0.00
vg^{ni}/vg^{ni}, $M(2)l^2$	♀	180	75.55	17.22	7.22
	♂	171	94.73	4.68	0.58
vg^{ni}/vg^{ni}, $M(3)w$	♀	171	8.19	24.56	67.25
	♂	169	44.97	44.97	10.06
vg^{nG}/vg^{nG}	♀	240	95.83	3.75	0.42
	♂	270	95.18	4.44	0.37
vg^{nG}/vg^{nG}, $M(2)l^2$	♀	300	49.33	31.33	19.33
	♂	282	49.29	36.88	13.83
vg^{nG}/vg^{nG}; $M(3)w$	♀	228	24.56	34.21	41.23
	♂	219	18.72	36.53	44.75

of the female and 92.51 per cent of the male $vg^{nw}/+$, $M(2)l^2$ individuals had notched wings, and all of the female and over 99 per cent of the male $vg^{nw}/+$, $M(3)w$ flies had scalloping of the wings.

The extreme expression of the heterozygous vg^{nw} in combination with the Minutes is also shown by a comparison of the individuals having only one with those having both wings involved. While practically all the control individuals with scalloping had only one wing affected, almost all those with $M(3)w$ had

both wings involved. Those with $M(2)l^2$ were intermediate with regard to the degree of expression, with approximately two-thirds of the scalloped-wing individuals showing both wings affected.

The degree and position of scalloping was similar in both of these alleles. In the heterozygotes with $M(2)l^2$ the scalloping of the wings was much like that observed among $vg/+$, $M(3)w$ individuals (fig. 2). However, flies heterozygous vg^{No2} and vg^{nw} with $M(3)w$ had an even greater degree of scalloping, with the wing frequently showing extreme incisions which reduced its area by nearly one-third.

vg^{ni} and vg^{nG}

The two lesser alleles of vestigial, vg^{ni} and vg^{nG}, were also tested in heterozygous combination with the two Minutes. In these cases the prolongation had no visible effect, and therefore tests were made to determine the influence of the Minutes on individuals homozygous for these alleles. For this purpose, each of the two alleles was transferred by crossing over into the chromosome carrying $M(2)l^2$, and at the same time cultures were prepared which had the allele of vestigial in homozygous combination with $M(3)w$. Control individuals were the regular stock cultures of vg^{ni} and vg^{nG}. Matings were made in the manner previously described, and development took place at 23°C.

The results of the crosses involving homozygous vg^{ni} are incorporated in table 2. Although no scalloping of the wings was observed in the control flies, the introduction of either of the Minutes resulted in scalloping in the homozygous vg^{ni} flies. The effect of $M(3)w$ in homozygous vg^{ni} individuals was more extreme than that of $M(2)l^2$, with regard to both penetrance (percentage of flies with scalloped wings) and degree of expression (relative proportion of those having both wings involved). It is of interest that the sex difference observed in most of the other tests is reversed here. Homozygous vg^{ni} with both $M(2)l^2$ and $M(3)w$ had a more extreme effect in females than in males. Only in the tests of $vg/vg/+$, $M(2)l^2$ was a similar sex difference observed. The amount and position of the scalloping of the wings were similar to those observed in scalloped $vg/+$, Minute individuals.

The results of the crosses involving vg^{nG} are also given in table 2. Among the homozygous vg^{nG} control flies some scalloping occurred—that is, approximately 4.2 per cent of the females and 4.8 per cent of the males were affected. However, there was a significant increase in penetrance with the introduction of either of the Minutes. In the $M(2)l^2$ combination approximately 51 per cent of both males and females had nicked wings. With $M(3)w$ there was a significantly still higher proportion of individuals with scalloped wings, 75.4 per cent of the females and 81.28 per cent of the males. In the control flies and those with $M(2)l^2$ the majority had only one wing involved, while the flies with $M(3)w$ showed a larger proportion of two-wing scalloping. The position and degree of scalloping of the wings were similar to those observed for vg^{ni}/vg^{ni}, Minute individuals.

The data presented indicate that the four alleles of vestigial also respond to the prolongation of the third larval instar associated with the Minutes, and that the increase in scalloping of the wings closely parallels the increased dura-

tion of this instar. The degree of response also parallels the known action of the alleles without the presence of the Minutes. Both of the extreme vestigial alleles responded to the influence of the prolongation associated with the Minutes with greater frequency and degree than did *vg*, while the Minute effect on the lesser alleles can be observed only if those alleles are homozygous.

Influence of temperature on the Minute effect

It has been reported (HARNLY 1936; STANLEY 1935) that the culturing of homozygous vestigial larvae at a temperature greater than 29°C results in a significant increase in the length of the adult wings. Moreover, HARNLY concluded that the temperature effective period is limited to a period of time during the third larval instar, the range depending upon the temperature used. In view of these findings, it seemed advisable to determine whether temperature has any influence on the frequency of scalloping in Minute, *vg*/+ individuals.

Eggs produced by homozygous vestigial females mated to *M*(2)*l*², *M*(3)*w*, and wild type males were collected in the manner previously described and were placed in bottles containing the banana-agar medium. The bottles were distributed among three temperature chambers, set at 23°, 28° and 30°C, respectively. The temperature control in the chambers was accurate to within ±1°C. After all the larvae had pupated, the bottles were placed in the 23° chamber until the adults had emerged.

The results, shown in graphic form in figure 5, indicate that with at least one of the temperatures used in the study a marked influence on the scalloping effect was obtained. In the control flies scalloping of the wings occurred in none of the females at any of the temperatures, in only a small number of the males raised at 23° and 28°, and in none of the males raised at 30°. In each temperature test, the number of controls which were examined exceeded the number of flies carrying *M*(2)*l*² or *M*(3)*w*. Among the 150 *vg*/+, *M*(2)*l*² females raised at 23°, 43.3 per cent had one wing, and 10 per cent had both wings nicked; among those raised at 28°, the respective frequencies were 35.6 and 8.5 per cent of 118 flies; but at 30° none of the 134 females had scalloped wings. The respective frequencies for one wing and two wings scalloped in the males were 40.6 and 35.0 per cent of 143, 37 and 20.7 per cent of 92, and 8.0 and 1.8 per cent of 112. Among the *vg*/+, *M*(3)*w* females raised at 23°, 48.3 per cent of 209 had one wing scalloped and 20.6 per cent had both wings scalloped; 63.4 and 12.2 per cent of 164 raised at 28° were likewise affected; but only one per cent of 102 raised at 30° were affected, and these had only one wing scalloped. A greater proportion of these males than females had scalloped wings but here also the 30° temperature suppressed the Minute action. The respective frequencies of the *vg*/+, *M*(3)*w* males were 32.4 and 58.5 per cent of 207 raised at 23°; 28.1 and 62.1 per cent of 153 raised at 28°; and 10.5 and 0.0 per cent of 134 subjected to 30°.

Although the suppression effect occurring at 30° was observed to influence both the two-wing and one-wing scalloping, there is the suggestion that in general the effect on the degree of expression is more complete than the effect

on the penetrance—that is, the two-wing scalloped condition is more responsive than the one-wing to the action of the 30° temperature.

The effects of temperatures of 23°, 28°, and 30° on the frequency of wing scalloping in the Minute, *vg*/+ flies appear to parallel the known effect of

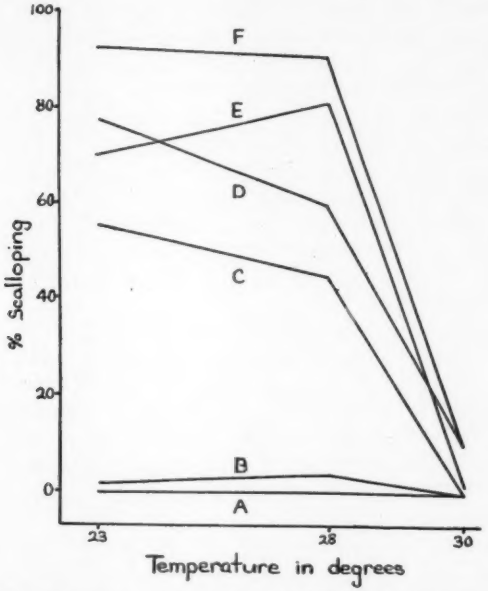


FIGURE 5.—Effect of temperature on penetrance in *Minute-vg* interaction. The % scalloping represents that proportion of flies having nicks in either one or both wings. The individual frequencies of one-wing and two-wing scalloping are given in the text. The temperature is centigrade. The graph marked A represents the control (*vg*/+) females; B, control males; C, *vg*/+, $M(2)l^2$ females; D, *vg*/+, $M(2)l^2$ males; *E*, *vg*/+, $M(3)w$ females; and F, *vg*/+, $M(3)w$ males.

temperature on homozygous vestigial flies. In homozygous vestigial individuals, no appreciable increase in the wing length is observed where larval development occurs at 23° and 28°, but if the temperature during larval development is raised to 30°, a significant increase in the wing length is noted. Similarly, in the case of wing scalloping in the Minute, *vg*/+ individuals, no significant difference in the frequency of wing scalloping was obtained at temperatures of 23° and 28°; but at a temperature of 30° a significant decrease in the frequency of wing scalloping was observed. Thus the effect of a temperature of 30° on either homozygous *vg* or *vg*/+, Minute is to cause an alteration in the development of the wings in the direction of the wild type phenotype. Since the frequency of wing scalloping in Minute, *vg*/+ individuals is affected by a "critical" temperature which is identical to that known to affect homozygous *vg*, it seems plausible to suggest that in each case temperature is acting on an identical process controlled by the *vg* gene.

Influence of crowding

In the first attempts to study the effects of the Minutes on *vg*/+ individuals, difficulty was encountered in obtaining consistent results. It was noted that the frequency of wing scalloping in Minute, *vg*/+ individuals coming from culture bottles yielding large numbers of progeny was less than among flies coming from bottles containing fewer progeny. These observations appeared to indicate that the nutrition of the larvae had a marked influence on the "scalloping" effect. Tests supported this inference.

Homozygous *vg* females were mated in vials to *Cy*/*M*(2)*l*^{2} or *M*(3)*w*/*In*(3*R*)*C* males. After 48 hours they were transferred to paper spoons and allowed to oviposit for 12 hours before they were transferred to fresh spoons. Eggs were collected at the end of each 12-hour period and were distributed in groups of 25, 50, and 100 to vials containing 10 cc of the regular banana-agar medium. Consequently, 25 larvae per vial had access to a greater amount of food than 50 larvae per vial, and 50 larvae to a greater amount than 100 larvae per vial. Development took place at 23°C.

Crowding of the larvae had a pronounced effect upon the scalloping frequency in the adults; the greater the number of individuals developing per vial, the lower the frequency of scalloping of the adult wings. Among 213 *vg*/+, *M*(2)*l*^{2} individuals with a mean number of 24.5 flies developing per vial, 30.3 per cent of the females and 86.7 per cent of the males were scalloped; with a mean number of 49.2 the respective frequencies were 9.4 and 28.7 of 207 flies; and with a mean number of 88, among 176 individuals there were no females and only 4.1 per cent of the males with scalloped wings. Similar results were obtained with *vg*/+, *M*(3)*w* individuals. Where the mean number of progeny per vial was 23.2, of 182 flies 77.6 per cent of the females and 97.9 per cent of the males were scalloped; with a mean number of 49.3 flies per vial the frequencies were 18.8 and 64.1 per cent of 101 flies; and with a mean number of 86.5 flies per vial the frequencies among 181 flies were zero and 21.3 per cent.

It was also noted that with an increase in the number of larvae developing per vial, a decrease in overall growth resulted. This was evidenced by the decreased size of the adults coming from heavily populated cultures. It was also shown by measurements of tibia length of the third leg of the adults. Although the details are not reported here, the tibia measurements indicated a significant decrease in tibia length with an increase in number of flies per vial.

DISCUSSION

Prolongation of the developmental time, effected by certain dominant Minute genes, increased the penetrance and degree of expression of heterozygous vestigial, causing scalloping of the wings of individuals which should otherwise have had normal wings. The observed increase in the frequency and degree of wing scalloping was closely correlated with the extent of prolongation of the third larval instar. *M*(3)*w*, which of the three Minutes used prolongs the third instar the most, caused the highest degree of scalloping. *M*(2)*l*^{2}, which

affects the third instar least, caused the least increase in wing modification. $M(1)n$ was intermediary in its effect.

The effect of a specific prolongation depends upon the dosage of *vg* and also upon the vestigial alleles that are used. In the tests on dosage effects, using a duplication of salivary chromosome regions 47–49 to increase the number of *vg* loci, the frequency and degree of scalloping were found to be greater among individuals having two vestigial genes and one normal allele than among those having one vestigial gene and two normal alleles. A comparison of the duplication and regular diploid individuals shows that the effect of prolongation was greatest among $vg/vg/+$ flies, less among $vg/+$ flies, and least among $vg/+/+$. As was the case with $vg/+$, the degree of effect of the prolongation in each of the duplication combinations varied with the degree of the increase in developmental time of the third instar. One can conclude therefore that both the increased frequency and degree of scalloping are closely associated with the dosage of the recessive vestigial gene in the genome, as well as with the extent of prolongation of the third larval instar of development.

In the case of the four alleles of *vg* studied here, the effect of prolongation of development associated with the Minutes was likewise expressed in increased scalloping of the wings. With each combination studied a greater prolongation caused a higher frequency and degree of wing scalloping. Moreover, the greatest effects of the Minutes occurred with vg^{nw} and vg^{No2}, which are the strongest alleles used in the study. With vg^{ni} and vg^{nG}, which normally show very little effect even when homozygous, there was no expression as a result of the Minute prolongation of the larval life of the heterozygotes; however, there was increased scalloping of the wings in the homozygotes in combination with the Minutes. The effect of prolongation on $vg/+$ was intermediate between that on the two stronger and the two weaker alleles. Thus, the increase in penetrance and degree of expression of these four alleles and vestigial itself associated with prolongation of the larval life parallels the strength of action of the alleles in a homozygous condition. All the alleles, however, were affected in the same direction—that is, away from the normal wing type.

Prolongation and the action of the vestigial gene

The data indicate that the prolongation effect of the Minutes is associated with the action of the *vg* gene and not with that of its normal allele. This fact is important in interpreting the nature of the action of *vg*.

None of the Minute genes in the presence of homozygous non-vestigial alone causes alteration in the wing phenotype. Only with the introduction of *vg* or one of its alleles into the genome is the scalloping effect observed. Moreover, the frequency and degree of wing scalloping vary with the dosage of *vg*. If prolongation of the larval life affected the normal allele of vestigial, one might expect the penetrance and expressivity to vary with the dosage of the normal alleles present in the genome. The scalloping effect should then be the same among $vg/+$ and $vg/vg/+$ individuals, since in each case only one normal allele is present in the genome. Actually, the data obtained demonstrate that there

is a significant difference in the frequency and degree of wing scalloping in $vg/+$ and in $vg/vg/+$ individuals, the greater penetrance being observed with the latter genome.

Furthermore, the alleles of *vg* fall into the same order with respect to penetrance under the prolongation influence of the Minutes that they do when homozygous. That is, the observable effects depend upon the strength of the allele used without respect to the normal allele. In Minute individuals with the genotypes $vg^{nw}/+$ and $vg^{No2}/+$ the effect of prolongation is most extreme. In $vg/+$ individuals the effect is less extreme. In the weaker alleles vg^{nG} and vg^{ni} scalloping occurs only upon prolongation of the larval life of the homozygotes —that is, when no normal allele is present. These results support the thesis that prolongation of the larval life influences the mode of action of the *vg* gene and its alleles other than the normal.

Temperature effect on Minute-vestigial interaction

The effect of the Minutes on heterozygous vestigial individuals is subject to control by temperature in the same direction as that found with homozygous *vg*, where at temperatures of 29° or greater a significant increase in the wing length results (HARNLY 1936; STANLEY 1935). No appreciable difference in the frequency and degree of scalloping of Minute, $vg/+$ individuals raised at 23° and 28°C was detected. However, when development took place at a temperature of 30° a significant reduction in both frequency and degree of scalloping of Minute, $vg/+$ individuals was observed.

Prolongation of the developmental time of $vg/+$ by the use of Minutes causes an action away from normal; while the added temperature control of 30° with the same genotype causes development towards normal. Homozygous *vg* developing at 30° also develops towards normal. And as with heterozygous vestigial, it seems probable that if the degree of change could be measured it would be discovered that the wings of homozygous vestigial flies would develop even farther away from normal under the prolongation influence of the Minutes and at a temperature of less than 30°. A suggestion that this would be true is found in the extreme expression of $vg/vg/+$ with the Minutes.

The parallel action of temperature on Minute, $vg/+$ and homozygous *vg* individuals indicates that in the two cases temperature is influencing parallel processes. The theoretical explanation might be made that the *vg* gene, when heterozygous, acts in a manner to produce some substance which is also elaborated in homozygous *vg* individuals. The amount of the substance produced in heterozygous *vg* individuals is not sufficient to cause wing scalloping. However, when the larval life of heterozygous *vg* individuals is prolonged as a result of the action of Minute genes, sufficient quantity of the substance is elaborated to cause scalloping of the wings. At a temperature of 30°C, either the process of production or the mode of action of the substance is modified such that wing differentiation takes place in the direction of the wild type phenotype, with little or no scalloping, even when the Minutes are present in the genome. The effect of prolongation, which allows the *vg* gene to act in the het-

erozygote, is suppressed by the high temperature, just as the high temperature tends to suppress the action of homozygous *vg*.

Nature of the vg gene action

The nature of the vestigial gene has been discussed by GOLDSCHMIDT (1935, 1938) and his collaborators, and by GREEN and OLIVER (1940). However, the results in the present study and the theory of gene action that has been reported by STERN (1943) suggest that the nature of action of *vg* should be reconsidered. According to one postulate, the *vg* gene, as well as its normal allele, should act to control the production of a wing growth substance, which must be present in a threshold quantity to guarantee normal development of the wings. The *vg* gene in homozygous condition is thus considered as being incapable of producing enough of the substance for normal differentiation of the wings. Some genes, such as *bb*, *ci*, w^e, are considered to act in the same direction as the normal allele but with a lesser efficiency or completeness. They would be placed in the category of hypomorphic genes (MULLER 1932).

The data presented in this study do not fit into the theory that *vg* is hypomorphic. If the gene for vestigial wings acts to elaborate a wing growth substance, it is not clear how scalloping could result in *vg*/+ individuals where, according to the theory, sufficient wing growth substance would be elaborated because of the action of the normal allele. Under the conditions of prolongation of larval life, one might expect an increase in the elaboration of such a growth substance produced by *vg*, and the adult wings would still be normal.

Apparently *vg* falls into MULLER'S category of antimorphic genes, in that *vg* acts to prevent normal differentiation of the wings. It is postulated here that *vg* controls the elaboration of a product or substance, which for simplification can be called the "*vg* substance," which acts to prevent the normal differentiation of the wings. In *vg*/+ individuals under normal conditions of development a subminimal amount of "*vg* substance" is produced; consequently the wings are normal. However, if the larval life, and more particularly the third larval instar, of *vg*/+ individuals is prolonged, the *vg* gene, which apparently is subject to control at that time, is able to produce a quantity of "*vg* substance" sufficient to cause some scalloping of the wings. The extent of this reaction appears to be proportional to the extent of prolongation of the third larval instar, as shown by the results obtained with the three Minutes used in this study. It is even possible by prolongation to cause one *vg* gene to act against two of its normal alleles.

The comparison of *vg*/+ and *vg*/*vg*/+ shows that the addition, by means of a small duplication, of an extra *vg* to the gene complex produces an effect away from normal rather than toward normal as would be expected with a hypomorphic gene. That is, increased dosage of the recessive *vg* gene causes an increase in the penetrance and degree of expression of *vg* as against its normal allele. In the tests made, the vestigial effect was least in *vg*/+/+ individuals, more in *vg*/+, and most in *vg*/*vg*/+, as shown by the frequency and degree of wing scalloping observed with individuals of those genotypes.

The prolongation effect for all the tested mutant alleles is in the same direction—that is, away from normal. The ability to elaborate the "*vg* substance" appears to vary with the different *vg* alleles, but their effect under conditions of prolongation is proportional to their effect when homozygous. That is to say, vg^{nw} and vg^{No2} when heterozygous cause an appreciable frequency of scalloping, even when the developmental time is not prolonged. This frequency of scalloping is markedly greater than that observed in heterozygous *vg*. Likewise, with increased prolongation of development the frequency and degree of scalloping among heterozygous vg^{nw} and vg^{No2} individuals are significantly greater than among heterozygous *vg* individuals. On the other hand, heterozygous vg^{nG} and vg^{ni} are not capable of producing sufficient "*vg* substance" even under the condition of prolongation to cause scalloping of the wings. However, when the larval life of homozygous vg^{nG} and vg^{ni} individuals is lengthened, sufficient "*vg* substance" is elaborated to cause scalloping of the wings. The quantity of "*vg* substance" elaborated here appears to be nearly equivalent to that elaborated by heterozygous *vg* individuals subjected to the same experimentation, except in the case of homozygous vg^{ni} males, in which the amount of "*vg* substance" seems to be appreciably less.

Thus, the vestigial gene can be considered antimorphic in action. Its below-threshold effect seems to be due not to its competition with the normal alleles for a limited quantity of substrate, but to a slower rate of development. The action of the *vg* gene can be best understood by assuming that it tends to prevent wing growth, but that its rate of differentiation (which can be considered a lower efficiency) is slower than that of its normal allele (GOLDSCHMIDT 1938; GOLDSCHMIDT and GARDNER 1942; GREEN and OLIVER 1940). This lower rate of reaction associated with *vg* can be compensated for by the addition of Minutes, which prolong the third larval instar and apparently also the developmental reactions occurring at that time. The prolongation tends to shift the dominance of *vg*, vg^{nw}, and vg^{No2}, the penetrance and degree of expression of these mutants varying in direct relation to the degree of prolongation of the third larval instar. Whether the effect is due to an actual retardation or to total prolongation of a developmental reaction is difficult to determine; however, BRAUN (1942) has suggested that retardation is associated with the change in the penetrance of vg^{no}. Minutes are not the only known genes which can affect the dominance of *vg*. Its penetrance can also be shifted by dominigenes (GOLDSCHMIDT 1935) and by other mutants (GARDNER 1942).

The rate of differentiation associated with vg^{ni} and vg^{nG} is of such low order that either gene in homozygous form will have only minor wing effects. Although prolongation of the third larval instar will not appreciably increase the dominance of either allele in competition with the normal allele, prolongation does increase the action of the homozygotes, in which an increase of the period of activity causes an increased wing destruction. In the homozygotes there is no competition with a normal allele, and prolongation apparently merely permits the gene to produce the substance in quantity sufficient to cause scalloping of the wings.

Larval nutrition also has a marked influence upon the elaboration of the

"*vg* substance." As a result of the crowding of Minute, *vg*/+ larvae a decrease in the frequency of scalloping results. This effect parallels the decrease in the growth of the larvae. It would seem that the elaboration of the "*vg* substance" is affected by the intermediary metabolism of the larvae, although the method of action is unknown. However, the results obtained with crowding show the need to control this possible means of influencing scalloping when the studies are made on the effects of dosage or temperature.

SUMMARY

A study has been made of the nature of the action of recessive vestigial as evidenced by the interaction of different dosages of *vg*, and of four of its alleles, with Minutes which prolong the duration of the third larval instar.

The frequency and degree of scalloping of the wings in flies *vg*/+ with *M*(2)*l*^{2}, *M*(1)*n*, or *M*(3)*w* were greater than in the controls. Scalloping is in general correlated with the increased duration of the third larval instar associated with each Minute.

Addition of an extra *vg*, by the use of a small duplication, increased the frequency and degree of scalloping both in controls (*vg*/*vg*/+) and in those carrying *M*(2)*l*^{2} or *M*(3)*w*. One *vg* expressed itself in competition with two normal alleles (*vg*/+/+) when Minutes were in the genome. With either Minute, *vg*/*vg*/+ flies had the most scalloping, *vg*/+ the next, and *vg*/+/+ the least. The effect away from normal was proportionally greater with *M*(3)*w*, which prolongs the third larval instar more, than it was with *M*(2)*l*^{2}.

Four alleles of vestigial were tested in combination with *M*(2)*l*^{2} and *M*(3)*w*. The two whose homozygotes are more extreme than homozygous *vg* also showed a higher degree of scalloping than *vg*/+ when the heterozygotes were combined with the Minutes. The two lesser alleles produced scalloped wings in combination with Minutes only when these alleles were homozygous.

At a temperature of 30°C, *vg*/+ with either Minute had no higher frequency or degree of scalloping than did the controls.

Crowding tends to counteract the effect of the Minutes.

It is postulated that *vg* and the four alleles, *vg*^{nw}, *vg*^{Nc2}, *vg*^{ni}, and *vg*^{nG}, are antimorphic genes which act to prevent wing development—that is, the action is contrary to that of the normal allele. In *vg*/+, the vestigial effect is below threshold due to the slower rate of development associated with *vg*. This can be compensated for by prolonging the third larval instar with Minutes, or by adding an extra *vg* to the genome. The two alleles *vg*^{ni} and *vg*^{nG} have a rate so low that prolongation of the development in heterozygous individuals does not change their dominance; but prolongation does cause the homozygotes to have wings that are scalloped. That is, the resulting development is in a direction opposite to normal.

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ON LETHAL MUTATIONS IN NATURAL POPULATIONS

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INTRODUCTION

DUBININ (1931) and DUBININ *et al.* (1934) found that natural populations of Drosophila contain tremendous proportions of autosomal lethal heterozygotes. This has been confirmed by STURTEVANT (1937a), DOBZHANSKY and QUEAL (1938), BERG (1941), OLENOV *et al.* (1939), MURETOV (1939), MAZING (1939), GERSHENSON (1941), and others.

Studies of lethals offer new perspectives for population genetics. The detection of lethals is easy and accurate; their incidence in populations is frequently very high; their variety is tremendous, amounting to hundreds of different genes; the total frequency of lethal mutations is also very large, up to one per cent or more per generation in the halploid chromosome set. Through investigations of lethals it is possible to study the roles played by the basic factors of population genetics: natural selection, mutation pressure, and automatic genetic processes (genetic drift). Starting in 1937, the writer, in collaboration with V. N. BELIAEVA, S. J. GOLDAT, N. D. DUSEEVA, E. N. KOPYLOVA, V. V. MANSUROVA, K. A. PANINA, E. D. POSTNIKOVA (Voronezh State University), and V. V. KHVOSTOVA, has made numerous experiments involving tens of thousands of crosses. The present communication reports only a part of the results obtained. The completion of the program will require several years of further work.

THE NATURAL MUTATION PROCESS IN THE SECOND CHROMOSOME OF NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER

The mutation frequencies were studied mostly through balancing wild chromosomes against a chromosome containing the dominant gene Curly (*Cy*) and two inversions. The populations studied came from Kutaisi, Caucasus (1939), Simferopol, Crimea (1939), and Sochi, Caucasus (1938). Two experiments were made with Simferopol flies. In the first, 498 wild chromosomes free of lethals were studied. Among the 476 strains carried as far as the third generation there were three lethals (0.63 ± 0.36 per cent). In the second experiment, the heterogeneity of the wild chromosomes with respect to the mutability was examined. Ten chromosomes free of lethals were taken, and 5603 crosses were made with them to study the mutation rates. The results are shown in table 1.

The data in table 1 suggest a heterogeneity among the chromosomes with respect to their mutability. The highly mutable chromosome No. 80 was examined twice, giving 2.02 ± 0.58 and 0.56 ± 0.25 per cent of mutations. Since the difference between the two experiments is not significant, the two experiments can be combined, giving 1.13 per cent of mutations. The only statistically significant difference, however, is between No. 219 and the first experi-

TABLE 1

The Simferopol, 1939, population. The frequency of spontaneous mutation in individual wild chromosomes, a repeated analysis of a highly mutable chromosome (No. 80), and the total mutation frequency.

WILD MALES (BY NO.)	28	107	277	219	270	98	4	281	280	80	80 (REPEAT)	80 (JOINTLY)	TOTAL WITHOUT NO. 80	1ST EXPERIMENT	GRAND TOTAL
Chromosomes studied	469	501	551	873	545	564	501	495	512	592	899	1491	5487	476	6978
Number of lethals	0	0	0	1	1	2	2	2	3	12	5	17	14	3	31
Frequency of lethals in percentage				0.11	0.18	0.35	0.39	0.40	0.58	2.02	0.56	1.13	0.25	0.63	0.44
Mean error				0.11	0.17	0.13	0.28	0.29	0.30	0.58	0.25	0.37	0.06	0.36	0.08

ment on No. 80. The mutation rate in the Simferopol population, then, is 0.44 ± 0.08 per cent.

The experiments on the Simferopol population were made in the laboratory. Five consecutive analyses were made, each analysis involving two crosses of the wild line with a laboratory one. The probability that the "wild" genotype as a whole was preserved, even leaving crossing over out of account, is $(\frac{1}{4})^3$ for the first analysis, $(\frac{1}{16})^3$ for the second, $(\frac{1}{64})^3$ for the third, etc. It is practically certain that in all the experiments the mutation rates were studied in an artificial genotype. To test the importance of the natural genotype and the natural environment, two experiments were made with the Kutaisi population. In the first of them, the mutation rate was studied in the gametes of males from nature, in the second in an artificial laboratory genotype. In the first experiment 790 chromosomes of 71 males were studied; 18 males were heterozygous for lethals brought in from the natural population; among 582 chromosomes of the remaining 53 males there were three lethals (0.51 ± 0.29 per cent). In the second experiment, ten strains were studied with the aid of the same method described above for the Simferopol population. The results are reported in table 2.

TABLE 2

The Kutaisi, 1939, population. The frequency of spontaneous mutation in individual wild chromosomes placed on a laboratory genetic background, compared with that in the genetic background of the natural population.

WILD MALES (BY NO.)	420	490	403	448	530	440	430	523	556	557	TOTAL	NATURAL GENOTYPE	GRAND TOTAL
Chromosome studied	500	510	463	503	543	563	594	546	508	469	5203	582	5785
Number of lethals	0	0	0	0	1	1	2	2	2	6	16	3	19
Frequency of lethals in percentage					0.18	0.18	0.34	0.37	0.39	1.28	0.31	0.51	0.33
Mean error					0.17	0.17	0.13	0.25	0.28	0.47	0.07	0.29	0.07

Taking the data at their face value, different mutation frequencies are indicated among the ten Kutaisi chromosomes examined. No statistically significant differences, however, are observed; neither is there a significant difference between the mutability in the natural genotype on the one hand and the laboratory one on the other. The mutation frequency in the total of 5785 Kutaisi chromosomes is 0.33 ± 0.07 per cent.

The mutation frequency in the Sochi population was studied in a mixed

TABLE 3

A summary of the data on the spontaneous mutability for lethals in the second chromosome of Drosophila melanogaster.

AUTHOR	POPULATION	YEAR	CHROMOSOMES EXAMINED	LETHALS OBTAINED	FREQUENCY IN %
MULLER	Laboratory	1928	17,572	69	0.40 ± 0.01
DUBININ	Simferopol	1939	6,978	31	0.44 ± 0.08
DUBININ	Kutaisi	1939	5,785	19	0.33 ± 0.07
DUBININ	Sochi	1938	1,324	6	0.45 ± 0.10
BERG	Nikitski Sad	1937	628	8	1.27 ± 0.42
BERG	Delizhan	1939	511	2	0.39 ± 0.27
BERG	Kashira	1940	1,016	5	0.49 ± 0.21
BERG	Serpukhov	1940	900	5	0.43 ± 0.25
OLENOV	Simferopol	1938	400	2	0.50 ± 0.30
OLENOV	Uman	1937	451	19	4.20 ± 0.94
OLENOV	Uman	1938	340	16	5.90 ± 1.20
OLENOV	Bobrov	1938	435	18	4.60 ± 1.00

natural-laboratory genotype. Six lethals were found in 1324 chromosomes; the frequency is 0.39 ± 0.04 per cent.

There is much evidence that natural populations are heterogeneous with respect to mutability. BERG (1942b) in the Kashira population and IVES (1943) in Florida found strains producing many lethals among less mutable strains. Similar observations have been made by DEMEREC (1937), TINIAKOV (1939), DUSEEVA (unpublished), and others. The mutable lines are apparently characterized by a general increase of the mutation frequencies, rather than by a high mutability of specific genes. The available data on the mutability of the second chromosome are summarized in table 3.

THE NATURAL MUTATION PROCESS IN THE SEX CHROMOSOME OF DROSOPHILA MELANOGASTER

MR. E. N. VOLOTOV attempted in 1935 to compare the mutation frequencies in the X chromosomes of different strains of the Gelendzhik (Caucasus) population. No mutations were found in 576 crosses, and the work on the problem was discontinued. Table 4 presents a review of the data in the literature bearing on the mutability of the X chromosomes in different populations.

The data on the mutability in the Florida population are reviewed in table 5.

TABLE 4

Frequencies of lethals in the X chromosomes of different wild strains.

AUTHOR	POPULATION	YEAR	CHROMOSOMES EXAMINED	LETHALS OBTAINED	FREQUENCY IN %
ZUITIN	Sukhumi	1938	2,039	24	1.04±0.21
DUSEEVA	Akhalcikh	1938	1,300	10	0.77±0.13
DEMEREC	Wooster	1937	1,266	8	0.63±0.15
DUCEEVA	Vladikavkaz	1938	1,544	8	0.51±0.18
DEMEREC	Formosa	1937	2,054	8	0.39±0.09
SAKHAROV	Nalchik	1937	5,169	18	0.34±0.08
ZUITIN	Vladikavkaz	1938	2,348	8	0.30±0.11
LOBASHOV	Vitebsk	1937	402	1	0.25±0.25
LOBASHOV	Kuibyshev	1937	506	1	0.19±0.19
Lobashov	Merv	1937	1,424	2	0.14±0.14
MAGRZHIKOVSKAIA and SAKHAROV	Samarkand	1941	4,416	5	0.11±0.05
TIMOFEEF-RESSOVSKY	8 lines	1940	68,221	95	0.14±0.02
DEMEREC	12 lines	1937	13,612	14	0.10±0.02

It may be seen that substrains of the Florida strain show an amplitude of variations of mutation rates comparable to that found in strains of different origin. The mean mutation rate in Florida is 0.41 per cent, in strains from different parts of USSR it is 0.40 per cent. According to the count of SHAPIRO (1938) and of BERG (1941), the average mutability of laboratory strains is 0.23 per cent. If we include the data of DEMEREC and TIMOFEEF-RESSOVSKY, the average mutability of the strains shown in table 4 turns out to be 0.22 per cent.

Comparison of wild and of laboratory strains indicates no difference in mutability between them. Lines with high and with low mutability occur in both groups. A review of the data on the mutability of whole populations for sex-linked lethals is given in table 6.

TABLE 5

Frequencies of lethals in the X chromosomes of different laboratory lines derived from the Florida strain.

AUTHOR	YEAR	CHROMOSOMES EXAMINED	LETHALS OBTAINED	FREQUENCY IN %
PLOUGH and CHILD	1937	172	2	1.10±0.80
DEMEREC	1937	2,108	23	1.09±0.15
SHAPIRO and VOLKOVA	1938	9,228	46	0.50±0.07
SHAPIRO and VOLKOVA	1938	9,228	22	0.24±0.05
SAKHAROV	1941	81,457	185	0.22±0.01
OLENOV	1938	2,397	5	0.21±0.08
ZUITIN	1938	8,614	14	0.16±0.04
BERG	1940	1,041	1	0.09±0.04
ZUITIN	1938	4,601	4	0.08±0.04

The data in table 6 indicate that populations may be characterized by different mutabilities. Unfortunately, the experiments have been done on an insufficient scale, and the differences observed are not statistically significant.

It may be noted that the rates of mutation in the second and X chromosomes do not correspond to their respective lengths: the rates are about equal while

TABLE 6

Frequencies of lethal mutations in the X chromosomes of different populations.

AUTHOR	POPULATION	YEAR	CHROMOSOMES EXAMINED	LETHALS OBTAINED	FREQUENCY IN %
ZUITIN and PAVLOVEC	Kutaisi	1937	460	6	1.34±0.55
ZUITIN and PAVLOVEC	Oni	1937	378	4	1.05±0.35
OLENOV	Uman	1937	838	10	1.20±0.38
BERG	Nikitski Sad	1937	1,206	10	0.83±0.26
ZUITIN	Sukhumi	1938	3,599	14	0.81±0.21
BERG	Serpukhov	1940	1,013	6	0.59±0.22
BERG	Kashira	1940	2,081	7	0.34±0.13
ZUITIN	Vladikavakaz	1938	1,520	2	0.13±0.09
BERG	Delizhan	1939	712	1	0.13±0.13
VOLOTOV	Gelendzhik	1934	576	0	—

the second chromosome is approximately 2.5 times longer than the X. It is possible that this non-correspondence is due to insufficient data.

ON DIFFERENT MUTABILITIES OF WHOLE POPULATIONS

As stated above, the data on the mutation rates for sex-linked lethals in different whole populations indicate, but do not prove, the existence of appreciable heterogeneity. Among the ten populations examined for mutation rates in the second chromosome (table 3), seven populations from remote parts of USSR with sharply different ecological conditions are identical in mutability. The Nikitski Sad (Crimea) population shows a high mutability, but the difference between it and the other seven populations is not statictically significant. Only the Uman and the Bobrov populations show significantly high mutabilities, and it is the comparison of these populations with others that is used by BERG (1941) as evidence of different mutabilities in different whole populations. Unfortunately, the problem is insufficiently worked out. It seems certain that mutability changes in the evolution of species. But the acceptance of this idea does not mean discovery of the phenomenon. To ascertain its existence may be difficult. In particular, because of the recent introduction of *Drosophila melanogaster* in the USSR and in North America, as well as because of the association of this species with man, the divergence of populations in respect to mutability may be relatively slight. The heterogeneity of populations with respect to mutability, in the sense that many populations contain lines with high and with low mutability, is, as we know, established. It is very

important to establish beyond a reasonable doubt that whole populations may also differ in mutability and that these differences are not accidental but express the evolutionary transformations of the populations.

THE MUTATION PROCESS AS AN ADAPTIVE CHARACTER OF THE SPECIES

The idea that the characteristics of the mutation process are an adaptive character of the species was formulated by DUBININ (DUBININ, *et al.* 1936) who wrote: "the direction and the rates of mutation of all the genes . . . are an adaptive character of the species which is subject to evolutionary change. The observed speed of the mutation process represents an equilibrium between the negative results of the mutation process (overburdening of the species with negative aberrations) and its necessity for the preservation of the evolutionary plasticity without which the species will perish with time." In a paper published in 1937, DUBININ discussed the problem in more detail. In 1938, there appeared papers by SHAPIRO and by STURTEVANT expressing similar ideas.

Recognition that the mutation process is an adaptive character leads to the idea that species and populations should have optimal frequencies and directions of mutability, creating most favorable combinations of the evolutionary plasticity with minimal lowering of the viability due to unfavorable mutations. The creation of optimal mutation frequencies requires that the evolution of the mutability be directed sometimes toward waning of the mutation rates, if they exceed the optimum, and sometimes toward waxing of the mutability, if it is below the optimum. Starting from similar ideas about the mutation process, R. L. BERG in a series of communications (BERG, *et al.*, 1941, 1942a, 1942b, 1943) expresses the opinion that there may be an interrelation between the evolution of the mutability and the genetic structure of the populations. According to BERG, the basic factor of the evolution of mutability is intergroup competition. If intergroup competition is stringent, the importance of adaptability increases, and selection favors most mutable genotypes. If a population is isolated, intergroup competition is lacking, selection is directed against the deleterious mutations which interfere with the adaptation of the species to the given environment and against variable genotypes, as a result of which the population is stabilized and the mutation rates decrease. BERG attempts to prove the validity of her hypothesis by comparing the mutation rates in five populations. The data in table 3, however, show that the hypothesis meets with difficulties. Seven of the populations have similar mutation rates, although they differ greatly in size as well as in the degree of isolation and in the whole complex of biotic factors. One of them (Kutaisi) lives under optimal conditions and is subdivided into a complex system of partially isolated micropopulations. Two populations (Simferopol and Sochi) occupy intermediate positions, while three (Kashira, Serpukhov, and Delizhan) are isolated colonies living under severe conditions.

ON THE RECESSIVITY OF THE LETHALS

The problem of the behavior of the lethals in heterozygotes is very important. The concentration of a lethal in the population is determined by the fre-

quencies of the mutations to and from the lethal, and by the action of selection. If the lethals are completely recessive, their elimination takes place only in homozygotes. The situation changes if the lethals have effects in heterozygotes. DOBZHANSKY and QUEAL (1938) examined the viability of lethal heterozygotes in *Drosophila pseudoobscura* and obtained an indication of a weak dominant effect of the lethals. Since the first experiments were defective because the experimental and control series were done at different times, DOBZHANSKY (1939) repeated the work. With the expected frequency of the wild type class being 33.33 per cent, the viability of heterozygotes for two lethals proved to be 33.11 ± 0.27, and of the control 33.59 ± 0.22 per cent. The difference, 0.48 ± 0.35 per cent, is not significant. In spite of this, DOBZHANSKY does not completely exclude the possibility that the differences in the concentration of lethals in different parts of America may be due to differences in the selection of the heterozygotes for these lethals.

We have examined the viability of heterozygotes for lethals obtained in

TABLE 7

The viability of 148 different lethals in heterozygotes.

POPULATION	NUMBER OF LETHALS	CROSS	TOTAL PROGENY	OBSERVED		CORRECTED		
				WILD TYPE	*Cy*	WILD TYPE	*Cy*	M_d/m_d
Kakhetia	33	*Cy*/*l*×Kakhetia/Kakhetia	27,173	13,787	13,386	13,858	13,315	71/30=2.06
1938		*Cy*/*l*×Florida/Florida	12,572	6,358	6,214	6,426	6,146	68/56=1.23
Control		*Cy*/Kakhetia×Kakhetia/Kakhetia	3,971	2,054	1,917			
Sochi, 1939	60	*Cy*/*l*×Sochi/Sochi	31,595	16,514	15,081	16,748	14,878	234/88=2.85
Control		*Cy*/Sochi×Sochi/Sochi	4,258	2,257	2,001			
Simferopol	55		26,777	All the lethals are recessive				

Kakhetia, Sochi, and Simferopol populations in 1938 and 1939. We have taken into account BERG's consideration, according to which a lower degree of dominance is expected in crosses of mutants with the wild type from the same population, and complete dominance in crosses of the mutants from wild populations with the laboratory strain Florida. Both types of crosses were made. Among the 148 lethals examined, all proved to be recessive in both crosses (table 7).

The same picture of the recessivity of lethals is found in the combined data of BERG (1941). For the 36 Kashira lethals, the control proportion of wild type is 52 ± 0.82 per cent, Curly 58 per cent; experimental: wild type 53.3 ± 0.27 per cent, Curly 46.7 per cent; the difference is not significant: $M_d = 1.3$, $m_d = 0.86$, $M_d/m_d = 1.5$. For the 32 Delizhan lethals, the control gave 50.6 ± 1.09 wild type and 49.4 Curly, the experiment 49.5 ± 0.26 per cent wild type and 50.5 Curly. The difference is also not significant: $M_d/m_d = 1.10/1.12 = 0.9$.

The papers of MAZING (1938, 1939) describe three series of experiments. Nine of the nineteen lethals studied from natural populations improved the viability of heterozygotes, the remainder were recessive. For another group of 32 lethals it is shown that 16 lethals decrease and four improve the viability

of heterozygotes. Among the 26 lethals from the Nikitski Sad population, eight decrease and two increase the viability of heterozygotes. The data shown in table 7 for *D. melanogaster*, as well as those of DOBZHANSKY (see above) for *D. pseudoobscura*, contradict the results of MAZING. Future investigations will clarify the reason for this contradiction.

A new, though indirect, method of investigation of the negative selection of lethal heterozygotes has been discovered through analysis of the relations between the frequency of the appearance of lethal homozygotes and the frequency of their spontaneous origin through mutation. The constant elimination of lethal homozygotes keeps each lethal at a certain low concentration in the population, thus preventing the overloading of the germ plasm by lethals above a certain level. However, since lethals arise in each generation through mutation, an equilibrium is reached at which the number of lethals eliminated in each generation through homozygosis (q^2) equals that arising by mutation (u). DOBZHANSKY (1936), using a method suggested by STURTEVANT, computed the frequency of lethal homozygotes in the Gelendzhik population according to the data of DUBININ *et al.* (1936). The computation is as follows. In 1933, the Gelendzhik population contained 8 per cent of second chromosome lethals (70 lethals in 877 chromosomes studied). It is evident that 0.64 per cent (0.08^2) of all zygotes will carry two lethals, one obtained from the father and one from the mother. In order to find out what proportion of the zygotes with two lethals will have two identical lethals, it is necessary to consider the experiment on the allelism of these lethals. To examine 51 lethals for allelism, 1,275 crosses were made ($51^2-51/2$). Identical (allelic) lethals were found in 38 crosses, or 2.98 per cent. Hence, 0.019 per cent of the zygotes in the population have two allelic lethals (0.0064×0.0298). A similar value for the 1935 collection is 0.032 per cent.

As shown in table 3, the usual mutation rate in the second chromosome is 0.40–0.50 per cent of lethals per generation. Hence, the frequency of mutation is 15–20 times greater than that of the elimination of the lethals through homozygosis. This discrepancy is observed in all populations so far studied. WRIGHT (in DOBZHANSKY 1939) pointed out that this discrepancy may be accounted for by a negative selection of the lethal heterozygotes, which could eliminate many lethals without having them first become homozygous. DOBZHANSKY and WRIGHT (1941) showed that in the Death Valley populations of *D. pseudoobscura* 12.59 per cent of the third chromosomes carry lethals. The frequency of homozygotes is 0.073 per cent and the mutation rate 0.297 per cent; in other words, the mutation rate is about 4.1 times greater than the elimination of lethals through homozygosis. The authors infer that the cause of this discrepancy may be either a local inbreeding taking place in the populations (F), or selection against lethal heterozygotes (s), or both. They arrive at a joint estimate of the value of these two possible factors ($F+s=0.013$), but are unable to establish either the relative roles of the two factors, or even the reality of them.

This shows how difficult is the experimental approach to the problem of the

effects of lethals in heterozygous condition. The selection against the lethal heterozygotes which must be assumed to account for the discrepancy in the data is very small. As quoted above, the control figure obtained in DOBZHANSKY's (1939) experiment on the recessivity of the lethals is 33.59 ± 0.22 per cent, and the experimental figure 33.11 ± 0.27 per cent. The difference, 0.48 per cent, is not statistically significant, and yet if taken at its face value, it is more than sufficient to account for the computed magnitude of the F+s. WRIGHT, DOBZHANSKY, and HOVANITZ (1942) reinvestigated this problem on a larger scale. The estimate of F+s is small for the populations of *D. pseudoobscura* which inhabit Mexico and Guatemala (0.0074); for the San Jacinto population it is 0.0201, and for Death Valley 0.0163. The authors point out that for a typical population ("station") on San Jacinto, a coefficient of 0.0177 may be due to about seven per cent of the matings in the population being between brothers and sisters. The inbreeding may take place during the eclipse phase in the numerical strength of the population. Nevertheless, the authors finally admit their inability to differentiate between the effects of inbreeding or of the negative selection of lethal heterozygotes.

BERG (1943) asserts categorically that the elimination of lethals in heterozygotes is tens of times greater than that in homozygotes. She believes that she can prove this assertion by her finding of the discrepancy between the elimination of the lethals in homozygotes and the mutation rate at the time (November) when the population of Nikitski Sad has begun to dwindle in numbers and the degree of isolation of separate foci (micropopulations) has begun to increase. She found the mutation rate of 1.27 ± 0.42 per cent, which is 36.1 times greater than the frequency of lethal homozygotes. No changes in the frequency of homozygotes however, are, expected at the time when the population begins to dwindle in numbers, if that population ceases to reproduce. At the stage of the development of the population examined by BERG, there is no reason to expect any deviation from the usual population structure. It is when the population, having passed through the eclipse stage, begins to increase in numbers that one may expect an increase in the number of lethal homozygotes. One must conclude that the problem remains open.

THE SEASONAL POPULATION CYCLE AND ITS RELATIONS TO THE GENE CONCENTRATIONS AND THE FREQUENCY OF LETHAL HOMOZYGOTES

When a large population carrying a great variety of genes undergoes a sharp reduction in numbers, the variety of the genes present in the population decreases. To be sure, the total frequency of the mutant genes need not change, However, if genes which were relatively rare in the large population are by chance preserved in the small one, their frequencies may increase. Assume, for example, that the initial population consisted of 100 individuals each heterozygous for a different lethal. The total concentration of lethals in the population is therefore 100 per cent, and the concentration of each particular lethal one per cent. Suppose now that this population dwindles to only ten individuals. The total concentration of lethals is still 100 per cent, but the

concentration of each lethal has increased from one to 10 per cent. When the diminished population starts to breed, a sharp elimination of the lethals will take place. Indeed, many lethal homozygotes are formed because of the increased frequencies of the individual lethals, and the frequencies of these lethals drop to the equilibrium levels. If the equilibrium concentration is the initial one,—namely one per cent,—the population will contain ten different lethal heterozygotes when it reaches the size of 100 individuals, each lethal having the frequency of one per cent.

After the process of elimination of lethals has taken place, the new cycle of the seasonal growth finds the population in the condition of undersaturation by lethals. The number of lethal homozygotes will be small and the mutation fre-

TABLE 8

Frequencies of second chromosome lethals in the Uman and Simferopol populations of Drosophila melanogaster.

AUTHOR	YEAR	POPULATION	MONTHS	LETHALS IN %
BERG	1937	Uman	July–August	14.6
OLENOV *et al.*	1937	Uman	September	22.6
OLENOV *et al.*	1938	Uman	August	21.9
OLENOV *et al.*	1938	Uman	September	29.8
OLENOV *et al.*	1938	Simferopol	August	20.6
DUBININ	1938	Simferopol	October	29.2

quency greater than the rate of elimination. In other words, the mutation pressure will increase the concentration of lethals. When the saturation point is reached, the mutation and elimination rates will be equal.

Among the populations of *Drosophila melanogaster* studied, the most usual concentrations of second chromosome lethals are around 20–25 per cent. Assuming the mutation rate to be about 0.5 per cent per generation (table 3), one may estimate that 40–50 generations are needed to have the frequency of lethals arise from zero to the level indicated. A single seasonal cycle, which involves about 10–15 generations in the southern parts of the USSR, may therefore cause appreciable shifts in the populations. The first indication of the existence of such shifts belongs to OLENOV *et al.* (1939) who found that in the population of Uman the concentrations of lethals grow from August to October (*cf.* table 8). OLENOV *et al.* interpreted the increasing concentrations between August and October as due to accumulation of mutants in a population which had previously been purged of its lethals, as indicated by the analysis of DUBININ and ROMASHOV (1932), but they failed to give a clear statement of the relationships between the mutation rates and the homozygosis for lethals.

Our data on lethal mutations collected over a period of years permit us to extend the experiments of OLENOV *et al.* In table 9 the data on the concentra-

tions of lethals are divided into four groups, those examined in July (12 populations), in September (two populations), October (three populations) and November (one population).

The data clearly show an increase in the concentration of lethals with the advance of the breeding season. Unfortunately, no analysis of this increase is

TABLE 9

Frequencies of second chromosome lethals in different months in different populations of Drosophila melanogaster

AUTHOR	YEAR	MONTH	POPULATION	CHROMOSOMES STUDIED	LETHALS FOUND	CONCENTRATION IN %
DUBININ *et al.*	1932	July	Kislovodsk	144	13	9.8
DUBININ *et al.*	1932	July	Mashuk	180	17	8.8
DUBININ *et al.*	1932	July	Essentuki	187	19	10.2
DUBININ *et al.*	1932	July	Vladikavkaz	161	32	19.9
DUBININ *et al.*	1932	July	Batumi	101	14	13.9
DUBININ *et al.*	1932	July	Piatigorsk	81	8	9.8
DUBININ *et al.*	1932	July	Erevan	102	16	15.7
DUBININ *et al.*	1932	July	Armavir	42	9	21.4
DUBININ *et al.*	1933	July	Gelendzhik	877	70	7.9
DUBININ *et al.*	1934	July	Gelendzhik	616	78	12.6
OLENOV *et al.*	1938	July	Simferopol	426	88	20.6
OLENOV *et al.*	1938	July	Uman	589	127	21.6
Mean for July				3,506	491	14.0
OLENOV *et al.*	1937	September	Uman	1,376	311	22.6
OLENOV *et al.*	1938	September	Uman	735	219	29.8
Mean for September				2,111	530	25.1
DUBININ	1938	October	Simferopol	510	149	29.2
DUBININ	1939	October	Simferopol	694	167	24.1
DUBININ	1939	October	Sochi	562	146	26.0
Mean for October				1,766	462	26.1
DUBININ	1939	November	Kutaisi	480	187	38.9

possible in the light of the relations of the number of generations and the mutation rates; such an analysis could be done only if adequate data on a single population on a certain year were available.[1]

[1] A section of DR. DUBININ's manuscript contains a discussion of data included in a table which was lost in transit. These data show that homozygosis for lethals occurs more frequently as the breeding season advances and the concentration of the lethals increases. Under the circumstances it seems advisable to delete this discussion.

THE RELATION OF MUTATION PRESSURE TO AUTOMATIC GENETIC PROCESSES

In very large and constantly breeding populations the distribution of lethals is determined entirely by mutation pressure and negative selection. In reality, the situation is more complex because the breeding cycles entail variations in the numerical strength and in the breeding system.

We have seen[2] that the populations contain many lethals each represented two or more times. As a rule, about half of the lethals are lethals found singly, while the other half consists of lethals found repeatedly. Thus, in the 1933 Gelendzhik population 29 out of 49 lethals occurred repeatedly, in the 1934 population 33 out of 88 were repeated; in the Ufa population 19 out of 43 were repeated; in the 1938 Simferopol population 33 out of 74, in the 1939 population 48 out of 128; and in the Kutaisi population 44 out of 88 lethals were repeated. The presence of so many repeated lethals in relatively small samples taken from these populations indicates that these are not random samples of lethals which may occur in the species. We are evidently sampling lethals that occur in the populations in relatively high concentrations. Concentrations of some of the lethals reach one, two, and even three per cent. Such high concentrations may be due either to a high mutability of the loci producing the respective lethals or to the accumulation of the lethals owing to automatic genetic processes.

DOBZHANSKY and WRIGHT (1941) analyzed the identity of lethals from various populations of *Drosophila pseudoobscura* and concluded that the differentiation of these populations with respect to the lethals which they contain is due mainly to automatic genetic processes. This conclusion is based on the lethals found in different populations failing to show a tendency toward a repeated occurrence in any one of these populations. However, in 1942, WRIGHT, DOBZHANSKY, and HOVANITZ reported a different result. Among the 152 lethals found singly within the various populations studied, only 18, or 11.9 per cent, were found repeatedly in different populations. Among the 25 lethals each of which was found twice within a population, seven, or 28 per cent, were repeated in the populations of different "stations" of the same "locality." Among the seven lethals each found thrice within a population, three, or 43 per cent, were repeated. The different results of their two experiments are accounted for by the authors by the differences in structure of the populations studied. The 1941 paper is concerned with populations of the Death Valley region which are relatively isolated from each other, while the 1942 paper is concerned with populations of a continuously inhabited territory on Mount San Jacinto.

We have made two series of preliminary experiments bearing on the problem under discussion. We have examined (1) the identity of lethals from different populations and (2) the identity of the lethals extracted from populations and those appearing in mutation experiments. If the distribution of lethals in

[2] This is another reference to the missing table.

populations is determined by the mutation pressure, then the distribution in all populations will show the same regularities. If, on the other hand, the distribution of lethals is influenced also by isolation, migration, and restriction of the population size, then different populations will show different distributions.

The experiments indicate that the distribution of lethals in different populations differs appreciably. The frequency of repeated lethals within a population is, on the average, 48 per cent, while the frequency of lethals repeated in different populations is only 19 per cent. It is, of course, conceivable that the mutation rates would be different in different populations. The mutation rates, however, are known to be alike in all the populations studied. The decisive data come from the Gelendzhik population in which the allelism of the lethals has been studied during two successive years. The frequency of repeated lethals within a year is equal to 48 per cent, while among the lethals found in different years only 27 per cent are repeated. The data permit us to compute the frequency of lethal homozygotes (that is, of zygotes containing the same lethal twice) that are produced in crosses between individuals from the same population as well as of those produced in crosses between individuals from different populations. The different populations studied were Ufa 1936, Simferopol 1938, Simferopol 1939, Sochi 1939, Gelendzhik 1933, and Gelendzhik 1934. The average for intra-populational crosses is 0.239 per cent, while the average for the inter-populational ones is only 0.057 per cent. This indicates that an important role is played in the distribution of lethals by the automatic genetic processes.

Nevertheless, some lethals occur repeatedly in different populations, and this suggests the important role of mutation pressure. The lethals found in different remote populations show a tendency to be found repeatedly within these populations as well. For example, there were six lethals in common between the Ufa and the Simferopol populations; three of these were found once each at Simferopol, while the three others belonged to those which showed high concentrations within the Simferopol population. One of them was found nine times at Simferopol (concentration 3.51 per cent). These data resemble those of WRIGHT, DOBZHANSKY, and HOVANITZ (1942) for *Drosophila pseudoobscura* but in our case the supposition that the frequent lethals have common origin is not applicable because of the great distances separating the populations concerned.

If some loci producing lethals have higher mutation rates than others, we must expect high frequencies of allelism in crosses between lethals found in the populations repeatedly as compared with lethals taken at random. We have crossed 24 lethals found repeatedly in the populations of Kutaisi (16 lethals), Sochi (three lethals), Ufa (one lethal), and Simferopol 1938 (four lethals), with 65 lethals taken at random from the 1939 population of Simferopol. Among the 1560 crosses, 12 crosses, or 0.79 per cent, contained allelic lethals, and eight, or 33 per cent, of the 24 repeated lethals had alleles among those found in the 1939 Simferopol sample. In another experiment, 97

lethals taken at random from the Kutaisi population were intercrossed with 15 lethals obtained in flies of Kutaisi origin by mutation. Among the 1455 crosses, only four or 0.28 per cent, contained alleles, and only two of the lethals obtained by mutation, or 13 per cent, had alleles among the lethals extracted from the natural population. Similarly, 32 lethals from the Simferopol 1939 population were crossed to 24 lethals obtained by mutation from flies of Simferopol origin; among the 768 crosses there were three alleles (0.39 per cent) and only three of the mutational lethals had alleles among the populational ones. Furthermore, one of the mutational lethals obtained in the Kutaisi material proved to be allelic to the populational lethal having the highest frequency in the Kutaisi population (3.44 per cent).

The above data indicate that some relatively highly mutable loci give rise to lethals found in populations as remote as those of Ufa, Simferopol, and Sochi. We know analogous situations among the visible mutants. Thus, the relatively highly mutable locus of yellow gives rise to mutant alleles found in many populations scattered over the tremendous expanse of territory of the USSR (Ukraine, Central Russia, Crimea, North Caucasus, Black Sea Coast of Caucasus, Transcaucasia, Volga region, Middle Asia). The similarities among the mutants found concealed in natural populations are due mainly to the similarities of the mutation process in these populations. The automatic genetic processes are the principal cause of the differentiation of the populations with respect to genes having no definite selective value.

The experiments presented here barely open a view of the perspectives of quantitative study of the genetic processes in populations. Further work will test the validity of the provisional hypotheses developed here.

DISCUSSION

The facts presented above show that disturbances of the genetic equilibrium occur in populations. Connected with them are rapid processes of saturation by mutants and of elimination of these mutants from the populations. These processes are related to the seasonal changes in the living conditions of the populations. The idea that some evolutionary processes may occur rapidly was expressed by DUBININ (1931) and DUBININ and ROMASHOV (1932). At present there can no longer be any doubt that the old notion that the evolutionary process is an extraordinarily slow one is invalid. It can be observed within a human lifetime.

The presence of lethals in populations may have some constructive significance in evolution.

Mutation pressure has an important negative influence in the life of a population. Most of the individuals of *Drosophila melanogaster* studied by us proved to be heterozygous for one, two, or more mutants. The unfavorable effects of the mutants in heterozygotes, as well as the appearance of mutant homozygotes, may seriously reduce the adaptive level of the population. The modern theories of the evolution of dominance and the ideas about mutation rates being

adaptive characters of the species in which they occur are based on a recognition of the importance of the mutation pressure in the life of populations. The importance of lethals in these phenomena is considerable, because lethals are a very frequent class of mutations. Moreover, changes in the breeding system may be a factor regulating the general mutability. Although a certain equilibrium between the mutation pressure and the elimination of homozygotes must exist with any breeding system, from wide intercrossing to strict self-pollination, a change in the breeding system may lead to inequalities in the numbers of homozygotes produced in different generations. Greater frequencies of homozygosis under certain conditions may become factors influencing the general mutation rates.

DUBININ and VOLOTOV (1941) have pointed out that, owing to the suppression of lethals by duplications, there must be a tendency toward the retention of duplications together with lethals. This would partly neutralize the recombination effect of crossing over. Homologous duplications and other suppressors of lethals, being favored by selection, would accomulate in the same chromosome in which the lethal lies. The lethals would therefore become factors of a peculiar intra-chromosomal evolution.

The locus of a lethal and a section of the chromosome in its immediate neighborhood are found in the population only in heterozygous condition. Since populations contain a multitude of lethals, the latter must play a certain role in the creation of heterosis.

Homozygosis for lethals leads to elimination from generation to generation of many chromosome sections lying in the proximity of the letbals. The lethals thus, are of importance in making populations essentially homozygous. Although heterosis is doubtless important in evolution, one must not forget that race and species characters which result from the evolutionary process are based on a homozygous gene complex. Evolution entails processes leading toward heterozygosis, toward creation of a system of intra-populational heterosis, as well as processes leading toward homozygosis. The importance of lethals in the phenomena of attainment of homozygosis is particularly great in populations containing inversions, because in this case large blocks of genes bound by the inversions may be eliminated. The lethals then become peculiar agents of intra-populational isolation. From among a large number of diverse alleles present at a given time in a large population, only relatively few alleles are eventually preserved. This process has been demonstrated through analysis of intraspecific chromosomal variability (DUBININ, SOKOLOV, and TINIAKOV 1937). Some inversions which now permeate the entire species area arose as single structural mutations; the fate of the chromosomes containing these inversions is easily followed, because they are marked by aberrations. But similar processes of replacement of the variety of the chromosomes now present in a population by the descendants of relatively few of these chromosomes are going on among chromosomes not marked by inversions, and among the variety of normal alleles of a given locus. In these processes of formation of homozygous gene complexes the role of lethals is not unimportant.

SUMMARY

The natural mutation rate for lethals was studied in 14,087 second chromosomes of *Drosophila melanogaster* from the populations of Kutaisi, Simferopol, and Sochi. The mutation rates proved to be 0.33 ± 0.07, 0.44 ± 0.08, and 0.45 ± 0.10 per cent, respectively. The mutation rates were studied in the natural genotype and under natural conditions, as well as in crosses with a laboratory strain in the Moscow laboratory. An analysis of 20 strains from the populations suggested the existence of a tendency toward different mutation frequencies.

It may be regarded as established that mutation rates may be identical in populations living in different environments, differing in the size of the breeding part of the population, and in the relations of the degrees of isolation and migration. In 35,114 second chromosomes from Florida, Simferopol, Kutaisi Sochi, Delizhan, Kashira, and Serpukhov 147 lethals have been obtained—that is, 0.42 ± 0.03 per cent.

The problem of recessivity of lethals was studied in 148 lethals from Kakhetia, Sochi, and Kutaisi. In 106,436 individuals the viability of lethal heterozygotes was compared with that of heterozygotes for a chromosome without a lethal. All the lethals proved to be recessive.

The idea of the importance of the seasonal cycle in the course of genetic processes in populations was expressed by DUBININ and ROMASHOV in 1932. The number of lethals eliminated through homozygosis equals, at equilibrium, the number of lethals arising through mutation ($q^2 = u$). Experimental analysis of the relations between the mutation rates and the frequencies of homozygosis for lethals, however, has shown that the expected equilibrium does not obtain in natural populations. The frequencies of homozygosis for lethals are as follows: Gelendzhik 1933—0.019 per cent, Gelendzhik 1934—0.031 per cent, Ufa—0.091 per cent, Simferopol 1938—0.034 per cent, Simferopol 1939—0.152 per cent, Kutaisi—0.248 per cent. The frequency of homozygosis for lethals is evidently much smaller than the mutation rate.

Concentrations of lethals and frequencies of homozygosis for lethals were studied for different parts of the seasonal cycle. For July, the mean concentration of lethals in ten populations was found to be 14.0 per cent, for September (four populations)—25.1 per cent, for October (four populations)—26.1 per cent, for November (one population)—38.3 per cent. The frequencies of homozygosis are: July—0.025 per cent, August—0.091 per cent, October—0.093 per cent, November—0.248 per cent. This shows a gradual dwindling of the discrepancy between the mutation and the elimination rates hand in hand with the numerical expansion of the populations and their saturation with lethals. This discrepancy is due to the lethals being rapidly eliminated during some and accumulated during other parts of the seasonal cycle. The accumulation of the lethals during the expansion of the populations, however, may be too rapid to be accounted for solely by the mutation pressure.

About half of the lethals found in the populations are represented by single heterozygotes, while the other half are found repeatedly; thus, for Gelendzhik

the figures are 72 and 62, respectively; for Ufa—19 and 24, for Simferopol—83 and 121, for Kutaisi—44 and 44. Some of the lethals reach concentrations of 1 per cent, 2 per cent, and even 3.5 per cent. On the other hand, crosses between lethals from different populations showed only 19 per cent of repeated lethals. The frequency of homozygotes obtained in crosses between lethals from the same population is 0.239 per cent, and in crosses between lethals from different populations 0.057 per cent on the average. This shows that the populations are differentiated owing to automatic genetic processes and that the concentration of lethals in them does not reflect solely the mutation rates. Nevertheless, the 0.057 per cent frequency of homoyzgosis for lethals obtained in crosses between most remote populations shows that the distribution of lethals is not accidental but is in part determined by the mutation pressures. This is confirmed by the fact that lethals found repeatedly in different populations show a tendency to have high concentrations within populations as well; these lethals arise at relatively mutable loci.

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ISOLATION BY DISTANCE UNDER DIVERSE SYSTEMS OF MATING

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INTRODUCTION

THE effects of restricted dispersion on the genetic properties of a continuous population have been treated mathematically in a previous paper (WRIGHT 1943a). The conclusions have been applied to the interpretation of observed local variability in a population of a plant, *Linanthus Parryi* (WRIGHT 1943b) and in one of an animal, *Drosophila pseudoobscura* (DOBZHANSKY and WRIGHT 1943). The mathematical treatment was based on the assumption of completely random union of gametes within each neighborhood and thus would rarely be strictly applicable to actual cases. The purposes of the present paper are to compare the effects of various systems of mating within continuous populations, and to present a more accurate method than before for estimating from data an important theoretical quantity, N, the effective size of population of a "neighborhood" in the sense discussed below.

It was postulated in the previous paper that a population of uniform density occupies either an indefinitely large area (area continuity) or a strip of indefinitely great length but of such narrow width that dispersion occurs across it within a single generation (linear continuity). Both uniparental and biparental reproduction were considered. In the former case, it was postulated that the locations of parents at some phase of the life cycle are distributed, relative to the corresponding locations of their progeny, according to a normal probability curve with a standard deviation σ, if there is linear continuity, or in a bivariate normal distribution with standard deviation σ, for both x and y coordinates of the parental locations (relative to their progeny) if there is area continuity. In the case of biparental reproduction the locations of the parents were assumed to be uncorrelated with each other. This is assumed to hold in the present paper except as qualified by self fertilization or brother-sister mating in excess of random.

A term is needed to designate the local population of which the parents may be considered as representative. Various terms were used in the preceding paper. "Panmictic unit" applies only if there is completely random mating locally and is thus not sufficiently general. "Parental group" is better but is somewhat awkward. An essential property of the population in question is that the individuals are neighbors in the sense that their gametes may come together. The term "neighborhood" is thus an appropriate one for this important unit.

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THE POPULATION NUMBERS OF NEIGHBORHOODS

To obtain numerical results, it was assumed in the previous paper that the mating system is equivalent to the random union of gametes produced by a population of N monoecious individuals, thus involving the proportion $1/N$ of self fertilization. This raises the question of the relation of N to the density of the population of mature individuals and the standard deviation of the parental distribution. Consider first the situation in a population of uniform density along a linear range. Assume that the location points of parents (x) relative to offspring may be represented sufficiently accurately by the normal distribution $y=(1/\sigma\sqrt{2\pi})\exp(-x^2/2\sigma^2)$. Let n be the number of potential parents in a strip of length 2σ. The density per unit distance is then $d=n/2\sigma$. The average length of the territory occupied by each individual may be written as follows

$$1/d = 2\sigma/n = \int_{x_i-\sigma/n}^{x_i+\sigma/n} dx \tag{1}$$

The chance that a particular gamete comes from a particular member of the parental generation at distance x_i is y_i/d. The chance that two uniting gametes came from the same individual is thus $\Sigma(y_i/d)^2$ where the summation applies to all individuals in the parental distribution. This is the expression to be equated to $1/N$. Making use of (1):

$$\frac{1}{N} = \sum_{x_i=-\infty}^{+\infty}\left[\frac{2\sigma}{n}\,y_i^2\int_{x_i-\sigma/n}^{x_i+\sigma/n} dx\right] = \frac{2\sigma}{n}\int_{-\infty}^{+\infty} y^2dx \text{ approximately}$$

$$= \frac{2\sigma}{n}\int_{-\infty}^{+\infty}\left[e^{-x^2/\sigma^2}/2\pi\sigma^2\right]dx$$

$$= \frac{1}{n\sqrt{\pi}}\int_{-\infty}^{+\infty}\left[e^{-x^2/2(\sigma/\sqrt{2})^2}/\sqrt{2\pi}(\sigma/\sqrt{2})\right]dx$$

$$= 1/n\sqrt{\pi} \tag{2}$$

$$N = \sqrt{\pi}\,n = 2\sqrt{\pi}\,\sigma d = 3.545\sigma d \tag{3}$$

Effective N is thus equivalent to the number of reproducing individuals along a strip $3.5\,\sigma$ long. About 92.4 per cent of the actual parents of individuals should fall within the range $\pm\sqrt{\pi}\,\sigma d$.

This method of relating N to σ and d can be extended to populations that are continuous over an area. Let $y=[1/2\pi\sigma^2]\exp[-(x_1^2+x_2^2)/2\sigma^2]$ be the distribution of the locations of parents relative to those of their offspring. Let n be the number of reproducing individuals in a square, 2σ on a side. The density per unit area is $d=n/4\sigma^2$. The average area occupied by an individual (coordinates x_1, x_2) may be written as follows:

$$\frac{1}{d} = \frac{4\sigma^2}{n} = \int_{x_2-\sigma/n}^{x_2+\sigma/n}\int_{x_1-\sigma/n}^{x_1+\sigma/n} dx_1dx_2 \tag{4}$$

The chance that two uniting gametes come from the same individual may be written as follows, using (4):

$$\frac{1}{N} = \frac{4\sigma^2}{n}\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} y^2 dx_1 dx_2 = \frac{1}{n\pi} \tag{5}$$

$$N = \pi n = 4\pi\sigma^2 d = 12.566\sigma^2 d \tag{6}$$

Effective N is equivalent to the number of reproducing individuals in a circle of radius 2σ. Such a circle would include 86.5 per cent of the parents of individuals at the center.

In an analysis of data from natural populations of *Drosophila pseudoobscura* (DOBZHANSKY and WRIGHT 1943), effective N was taken as equivalent to the breeding population in a circle of such radius ($\sqrt{2}\sigma$) that the volume of a cylinder erected upon it would equal that of the bivariate normal distribution with $\sigma_{x_1} = \sigma_{x_2} = \sigma$ and central ordinate equal to the height of the cylinder. This does not do justice to the dispersive effect of the more extreme parent-offspring distances. The estimate of effective N for a given parental standard deviation should be just twice that arrived at in this way. As the estimate in this application could at best be considered as giving only the order of magnitude, the effect of this correction is not very important.

The distribution of location points of grandparents relative to those of their grandchildren is compounded of their distributions about the location points of the parents and the similar distribution of these about the individuals in question. Thus the variance of the grandparental distribution is twice that of the parental distribution and its standard deviation is $\sqrt{2}\sigma$. The standard deviation for ancestors of generation K is similarly $\sqrt{K}\sigma$. The effective size of the population from which ancestors of generation K are taken is $\sqrt{K}N$ if the range is linear but KN if it extends in all directions.

CASE 1. MONOECIOUS POPULATIONS WITH EQUAL DISPERSION FROM MALE AND FEMALE PARENTS

This case was considered in the previous paper, subject to the postulate that union of gametes from the neighborhood is completely random (apart from the differential weighting of probabilities by distances of parental from offspring location). This implies self fertilization at the rate 1/N, a highly arbitrary postulate. It is desirable to determine the consequence under any specified percentage of self fertilization.

Let N be the effective population number in neighborhoods.

Let q be the gene frequency in a neighborhood.

Let h be the proportion of self fertilization.

The following quantities are relative to the population from which ancestors of generation K were drawn.

Let N_K be the effective population number.

Let q_K be the average gene frequency.

Let S be the correlation between gametes from the same individual.

Let D be the correlation between gametes from different individuals from the neighborhood, which unite or contribute to adjacent individuals. Let D_X be that between gametes from different individuals from the Xth ancestral generation—weighted according to their likelihood of contributing to the $(X-1)$st ancestral generation.

Let E be the correlation between gametes which contribute to adjacent individuals. Let E_X be the correlation between gametes from the Xth generation which contribute to the $(X-1)$st ancestral generation.

Let F be the correlation between uniting gametes. This is the inbreeding coefficient relative to the population of size N_K. It must be distinguished from the inbreeding coefficient relative to the neighborhood, which is $[h-(1/N)]/[2-h-(1/N)]$ from (16) below ($K=1$, $\Sigma t_A=0$) or from (16) and (18) by the formula $(F-E)/(1-E)$, Σt_A with $(K-1)$ terms.

Let $a(=\sqrt{1/2(1+F)}$, WRIGHT 1921) be the path coefficient relating zygote to one of the gametes which united in its production.

Let $b(=\sqrt{(1+F')/2}$, where F' is the value of F in the preceding generation, WRIGHT 1921) be the path coefficient relating gamete to the zygote which produced it. The compound path coefficient relating a gamete to one of the two from which it traces a generation earlier is $ba'=1/2$ where a' is the value of a in the preceding generation.

It will be assumed that the same population structure has continued indefinitely. Under this assumption primes may be dropped.

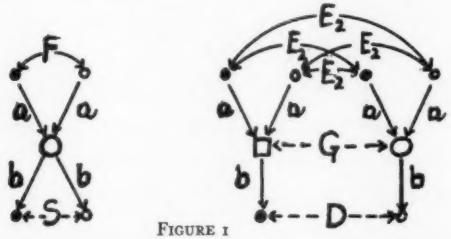

FIGURE 1

The following equations may be written:

$$E = \frac{1}{N} S + \frac{N-1}{N} D \qquad \text{by definition} \qquad (7)$$

$$F = hS + (1-h)D \qquad \text{by definition} \qquad (8)$$

$$S = b^2 = (1+F)/2 \qquad \text{from fig. 1} \qquad (9)$$

$$D = 4b^2a^2E_2 = E_2 \qquad \text{from fig. 1} \qquad (10)$$

Similarly $D_X = E_{X+1}$ by analogy with (10) (11)

Consider first the case of area continuity in which $N_K = KN$

$$E_X = \frac{1}{XN} S + \left[1 - \frac{1}{XN}\right] E_{X+1} \qquad (12)$$

$$\text{Thus } E = \frac{1}{N}S + \frac{N-1}{N}E_2$$

$$E_2 = \frac{1}{2N}S + \frac{2N-1}{2N}E_3$$

$$E_3 = \frac{1}{3N}S + \frac{3N-1}{3N}E_4$$

$$E_K = 0$$

$$E = \frac{S}{N}\left[1 + \frac{1}{2}\left(\frac{N-1}{N}\right) + \frac{1}{3}\left(\frac{N-1}{N}\right)\left(\frac{2N-1}{2N}\right)\cdots\right] \qquad (13)$$

The series in brackets, with K-1 terms, will be designated Σt_A. Formulae for its approximate evaluation were given in the preceding paper (formulae 22 and 27). Its limiting value for $K = \infty$ is N.

$$E = S\sum t_A/N \qquad \text{from (13)} \quad (14)$$

$$D = S(\sum t_A - 1)/(N-1) \qquad \text{from (7) and (14)} \quad (15)$$

$$F = [h(N-1) + (1-h)(\sum t_A - 1)]/[(N-1) + (1-h)(N - \sum t_A)]$$
$$\text{from (8), (9), (15)} \quad (16)$$

$$S = (N-1)/[(N-1) + (1-h)(N - \sum t_A)] \qquad \text{from (9) and (16)} \quad (17)$$

$$E = (N-1)\sum t_A/N[(N-1) + (1-h)(N - \sum t_A)]$$
$$\text{from (14) and (17)} \quad (18)$$

$$D = (\sum t_A - 1)/[(N-1) + (1-h)(N - \sum t_A)]$$
$$\text{from (15) and (17)} \quad (19)$$

The amount of differentiation among neighborhoods within the comprehensive population N_K can be found from a formula given in WRIGHT 1943a. It was shown there that if there is random mating within each of a number of populations, the variance of the values of q of these populations is given by the formula $\sigma_q^2 = q_K(1-q_K)F$, using the symbols defined here. In the present paper, random mating within neighborhoods is not assumed. However, E is defined in such a way that it would be the coefficient of inbreeding (F) if there were random mating in the last generation.

$$\sigma_q^2 = q_K(1-q_K)E \qquad (20)$$

This formula may be derived in another way. The allele present in a gamete taken at random from a neighborhood may be considered to be determined jointly by the gene frequency (q) of the latter and an uncorrelated deviation. The degree of determination is the square of the correlation between gametic value and q. It gives the portion of the variance of gametes, $q_K(1-q_K)$, for which differentiation of neighborhoods, measured by σ_q^2, is responsible. But the correlation (E) between pairs of gametes taken at random from neighborhoods is

also given by this same squared correlation. Thus $E = \sigma_q^2/q_K(1-q_K)$ in agreement with (20).

If self fertilization occurs wholly at random at all times ($h = 1/N$), we have the case treated in the preceding paper (formula 21, WRIGHT 1943a).

$$E = F = \sum t_A/(2N - \sum t_A) \qquad \text{from (18), } h = 1/N \quad (21)$$

If there is no self fertilization ($h = 0$), the result differs little, unless N is very small.

$$E = (N-1)\sum t_A/N(2N - \sum t_A - 1) \qquad \text{from (18), } h = 0 \quad (22)$$

Under exclusive self fertilization ($h = 1$) the amount of differentiation of neighborhoods is the same as that relative to two alternatives under any form of uniparental reproduction. The result agrees with formula (37) of the preceding paper.

$$E = \sum t_A/N \qquad \text{from (18), } h = 1 \quad (23)$$

The correlation between genotypes from a neighborhood (assuming additive effects) is independent of h in Case 1. The quantity, to be called G, is equal to D/b^2 as may be seen from figure 1.

$$G = D/b^2 = (\sum t_A - 1)/(N-1) \qquad (24)$$

Analogous results can be obtained for the case of linear continuity. As brought out in the previous paper, it is necessary merely to substitute the following series Σt_L for Σt_A. The approach to the limiting value N as the number of terms is increased is much more rapid.

$$\sum t_L = \left[1 + \frac{1}{\sqrt{2}}\left(\frac{N-1}{N}\right) + \frac{1}{\sqrt{3}}\left(\frac{N-1}{N}\right)\left(\frac{\sqrt{2}\,N-1}{\sqrt{2}\,N}\right)\cdots\right] \qquad (25)$$

The conclusions reached on the relation of effective N to density of population (d) and standard deviation of parental distances (σ), assuming random mating within neighborhoods, may seem to require reconsideration in this case in which a specified amount (h) of self fertilization is assumed. The quantity $1/N$, however, may be defined as the amount of self fertilization that there would be if there were random union of gametes in the neighborhood in the last generation and is independent of the amount of self fertilization that there actually is or has been. The chance that two gametes that enter into the production of two adjacent individuals (instead of two uniting gametes) come from the same individual may be written $1/N = \Sigma(y/d)^2$, leading to formulae (3) and (6).

CASE 2. PERMANENT PAIRS

The simplest system of mating with separate sexes is that in which reproduction is wholly by permanent pairs and the amount of dispersion of males and females is the same. The analysis is somewhat similar to that in the preceding case if the pair is treated as the unit and located at the point at which

pairing first occurs. The treatment of individuals as located at the points at which they began their careers as fertilized eggs (or at any later phase) would be complicated by a correlation between the parental localizations relative to those of offspring, beyond that which may be due to a tendency to brother-sister mating. As before, correlations are relative to a population of specified size (KN_P).

Let $N_P(=N/2)$ be the number of pairs in a neighborhood.

Let h here be the proportion of brother-sister mating.

Let C be the correlation between gametes produced by siblings.

Let D be the correlation between gametes of mated or adjacent non siblings, and D_X that between gametes of non siblings of ancestral generation $(X-1)$ relative to pairs.

Let E be the correlation between gametes of adjacent individuals and E_X be that between gametes of individuals belonging to ancestral generation $(X-1)$ relative to pairs.

Let F be the correlation between uniting gametes (the inbreeding coefficient).

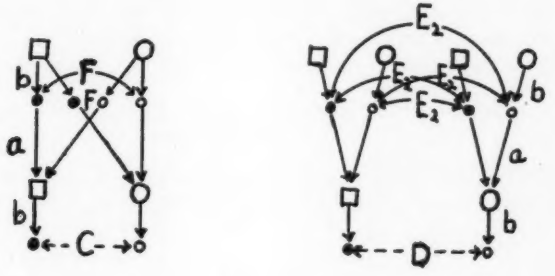

FIGURE 2

From inspection of figure 2

$$C = b^2a^2(2b^2 + 2F) = (1 + 3F)/4 \tag{26}$$

$$D = 4b^2a^2E_2 = E_2 \tag{27}$$

$$D_X = 4b^2a^2E_{x+1} = E_{x+1} \tag{28}$$

$$E = C/N_p + E_2(N_p - 1)/N_p \qquad \text{by definition and (27)} \tag{29}$$

$$E_X = C/XN_p + E_{x+1}(XN_p - 1)/N_p \qquad \text{by analogy} \tag{30}$$

$$E_K = 0 \qquad \text{by definition of } KN_p \text{ as the population of reference} \tag{31}$$

$$E = C\sum t_A/N_p \qquad \textit{cf.}\ (13), (14) \tag{32}$$

$$D = (N_pE - C)/(N_p - 1) = C(\sum t_A - 1)/(N_p - 1) \qquad \text{from (29), (32)} \tag{33}$$

$$F = C[h(N_p - 1) + (1 - h)(\sum t_A - 1)]/(N_p - 1)$$

by definition and (33) (34)

$$F = [h(N_p - 1) + (1 - h)(\sum t_A - 1)] / [(N_p - 1) + 3(1 - h)(N_p - \sum t_A)] \quad \text{from (26), (34)} \quad (35)$$

$$E = (N_p - 1)\sum t_A / \{N_p[(N_p - 1) + 3(1 - h)(N_p - \sum t_A)]\}$$

from 32, 26, 35 (36)

The amount of variation of gene frequency among neighborhoods is the same as that given in (20), $\sigma_q^2 = q_K(1-q_K)E$.

If brother-sister mating occurs at random at all times within neighborhoods, $h = 1/N_P$

$$E = \sum t_A / [4N_p - 3\sum t_A] \quad \text{from (36), } h = 1/N_P \quad (37)$$

A case which approximates that in a continuous human population of low mobility is that in which $h = 0$ (no brother-sister mating).

$$E = (N_p - 1)\sum t_A / [N_p(4N_p - 3\sum t_A - 1)] \quad \text{from (36), } h = 0 \quad (38)$$

With exclusive brother-sister mating ($h = 1$) all lines become homallelic, and the differentiation among neighborhoods becomes the same as under uniparental reproduction, except that pairs, instead of individuals, must be taken as the units.

$$E = \sum t_A / N_p \quad \text{from (36), } h = 1 \quad (39)$$

The correlation between random zygotes from the same neighborhood, assuming no dominance or factor interaction, is given in this case by E/b^2.

$$G = (N_p - 1)\sum t_A / \{N_p[(N_p - 1) + (1 - h)(N_p - \sum t_A)]\}. \quad (40)$$

In the case of a population with a linear range, it is necessary merely to substitute Σt_L for Σt_A in the preceding formulae.

The relation between effective number of pairs, standard deviation (σ_p) of distances between parent-offspring mating sites, and density of the pairs (d_p) can be found as in case 1.

$$N_p = 2\sqrt{\pi}\,\sigma_p d_p \quad \text{with linear continuity} \quad (41)$$

$$N_p = 4\pi\sigma_p^2 d_p \quad \text{with area continuity} \quad (42)$$

Since $N = 2N_p$ and $d = 2d_p$ and $\sigma = \sigma_p$ (if individuals are located at the site of first mating), formulae (41) and (42) apply to individuals by dropping all subscripts. They become identical with (3) and (6).

CASE 3. SEPARATE SEXES AND RANDOM MATING

Assume that males and females are both distributed uniformly but not necessarily with the same density over either an extensive linear range or an extensive area, that each offspring is produced by a separate random mating,

and that there is equal dispersion of sons and daughters. Let N_m and N_f be respectively the effective numbers of mature males and females per neighborhood. The diagrams below represent three of four possible relations between gametes of mated or adjacent individuals.

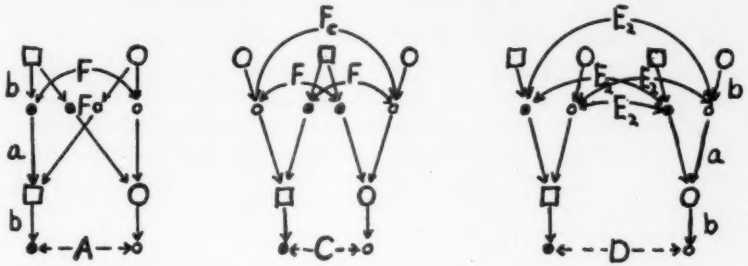

FIGURE 3

All correlations are assumed to be relative to a population of specified size $K(N_m+N_f)$.

Let A be the correlation between gametes produced by full brother and sister.

Let B and C be the correlations between gametes produced by half brothers and sisters with common mother and father, respectively.

Let D be the correlation between gametes of mated or adjacent individuals without a common parent, and D_x that between gametes of non-siblings of ancestral generation $(x-1)$ relative to matings.

Let $E=F$ be the correlation between gametes of mated or adjacent individuals and E_x that between gametes of individuals of ancestral generation $(x-1)$ relative to matings.

Let F_C be the correlation between gametes of individuals that mate at some time with the same individual.

Assuming random mating, the probabilities of the four cases and their contributions to E are readily determined.

$$E = [A + (N_m - 1)B + (N_f - 1)C + (N_m - 1)(N_f - 1)D]/N_mN_f \tag{43}$$

$$A = b^2a^2(2b^2 + 2F) = (1 + 3F)/4 \tag{44}$$

$$B = C = b^2a^2(b^2 + 2F + F_C) = (1 + 5F + 2F_C)/8 \tag{45}$$

$$D = 4b^2a^2E_2 = E_2 \tag{46}$$

$$D_x = 4b^2a^2E_{x+1} = E_{x+1}. \tag{47}$$

Collecting terms and multiplying N_m and N_f by X,

$$E_X = \left(\frac{N_m + N_f}{XN_mN_f}\right)\left(\frac{1 + 5F + 2F_C}{8}\right) + \left[1 - \left(\frac{N_m + N_f}{XN_mN_f}\right)\right]E_{X+1} - \left(\frac{F + F_C - 2E_{X+1}}{2X^2N_mN_f}\right) \qquad (48)$$

In the last term the numerator does not include population numbers and is twice the difference between two quantities that are both less than one and nearly the same if X is small; the denominator involves X^2. This term must always be much smaller than any of the others. It will be assumed that it can be ignored.

It will be convenient to write N′ for $N_mN_f/(N_m+N_f)$.

$$E = [(1 + 5F + 2F_C)/8N'] + (N' - 1)E_2/N' \quad \text{approximately}$$

$$E_2 = [(1 + 5F + 2F_C)/16N'] + (2N' - 1)E_3/2N' \quad \text{approximately}$$

$$E_X = [(1 + 5F + 2F_C)/8XN'] + (XN' - 1)E_{X+1}/XN'$$

$$E_K = 0$$

$$E = [(1 + 5F + 2F_C)/8N']\sum t_A. \qquad (50)$$

The correlation F_C cannot differ appreciably from F (and E) although theoretically slightly smaller. Substituting E for both F and F_C, the following approximate solution can be obtained.

$$E = \sum t_A/(8N' - 7\sum t_A). \qquad (51)$$

Special cases of interest are that in which $N_m=N_f=N/2$, $N'=N/4$, and that in which N_f is indefinitely large in comparison with N_m with the consequence that $N'=N_m$.

The amount of differentiation among neighborhoods is given as in other cases by formula 20, $\sigma_q^2=q_K(1-q_K)E$.

Again it is necessary merely to substitute Σt_L for Σt_A to obtain the formula applicable to a linear range.

To relate N_m and N_f to the densities d_m and d_f of males and females respectively and to the standard deviation of parent-offspring distances $\sigma_m=\sigma_f=\sigma$, let $y=(1/\sigma\sqrt{2\pi})\exp(-x^2/2\sigma^2)$ be the distribution of parents to offspring in a linear range. Let n_m and n_f be the numbers of males and females in a strip 2σ long. The mean space occupied by males is $1/d_m=2\sigma/n_m$ and by females is $1/d_f=2\sigma/n_f$. The chance that adjacent individuals have a common father is

$$\frac{1}{N_m} = \sum_{x=-\infty}^{+\infty}(y/d_m)^2 = \sum_{x=-\infty}^{+\infty}\frac{1}{d_m}y^2\int_{x-\sigma/n_m}^{x+\sigma/n_m}dx$$

$$= 1/(2\sqrt{\pi}\,\sigma d_m) \qquad (52)$$

Thus $N_m = 2\sqrt{\pi}\,\sigma d_m = 3.5\sigma d_m$ (53)

Similarly $N_f = 2\sqrt{\pi}\,\sigma d_f = 3.5\sigma d_f$.

The result is the same per individual as in cases 1 and 2. This is also true for area continuity.

$$N_m = 4\pi\sigma^2 d_m = 12.6\sigma^2 d_m \qquad (54)$$
$$N_f = 4\pi\sigma^2 d_f = 12.6\sigma^2 d_f.$$

CASE 4. DISPERSION BY GAMETES OF ONLY ONE SEX

The assumption that the distribution of parent-offspring distances is the same for both parents often does not apply. Thus among plants, dispersal of seed may be negligible in comparison with that of pollen. The assumption of different variances for male and female parents leads unfortunately to great algebraic complexity. It must suffice here to consider the limiting case in which all dispersal is through one parent (taken as the male). For simplicity, we shall assume all individuals to be hermaphrodites but with self fertilization occurring to any specified extent. The population will be assumed to be of uniform density, either along a strip of indefinitely great length, negligible width, or over an area of indefinite extent in all directions. In the former case, the distribution of pollen parent about progeny (and ovule parent) will be assumed to be univariate normal with standard deviation σ, apart from excess (or defect) of the middle class due to excess (or defect) in amount of self fertilization relative to that under random fertilization. Letting n be the number of individuals in a strip 2σ long, the average density is $d = n/2\sigma$ and the average interval between individuals is $2\sigma/n$. Let N be the effective number of individuals in the neighborhood that function as pollen parents, without taking cognizance of excess self fertilization. Then the chance of self fertilizat on under random union is $(1/N) = (2\sigma/n)y_0$ where y_0 is the midordinate of the normal distribution $y = (1/\sigma\sqrt{2\pi})\exp(-x^2/2\sigma^2)$. It is assumed that N is sufficiently large that the middle class of the distribution can be represented reasonably well by the product of midordinate into class range. Thus

$$1/N = 2\sigma/n\sigma\sqrt{2\pi} = (1/n)\sqrt{2/\pi}$$
$$N = n\sqrt{\pi/2} = \sqrt{2\pi}\,\sigma d = 2.50\sigma d. \qquad (55)$$

In the case of area continuity, we assume that the distribution of pollen parents is bivariate normal relative to ovule parents and progeny, $y = (1/2\pi\sigma^2)\exp[-(x_1^2+x_2^2)/2\sigma^2]$. Letting n be the number of individuals in a square 2σ on a side, the average density is $d = n/4\sigma^2$, and the average space per individual is $4\sigma^2/n$. Defining N as above, the chance of self fertilization under random mating of gametes in the neighborhood is $4\sigma^2 y_0/n$.

$$1/N = 4\sigma^2/(n(2\pi\sigma^2)) = 2/\pi n$$
$$N = \pi n/2 = 2\pi\sigma^2 d = 6.28\sigma^2 d. \qquad (56)$$

N is thus equivalent to the number of individuals in a circle of radius $\sqrt{2}\sigma$ in this case. It is half as great as in the case of an equally dense population of hermaphrodites with the same amount of dispersion of both male and female

gametes as of male gametes in this case. If the variance of pollen dispersal is twice that of the preceding cases but d is the same, formulae (55) and (56) become the same as (3) and (6), respectively.

Let r be the proportion of the pollinations that may be considered as at random from the neighborhood and $(1-r)$ the excess self fertilization. Assuming that N is at least 2, the variance of pollen parents in any direction is $r\sigma^2$ (area continuity), since self fertilization contributes nothing. The total proportion of self fertilization is $h=(r/N)+(1-r)$.

The correlation (E) between ovules and pollen grains that contribute to adjacent zygotes and that (F) between ones that unite may be analyzed as indicated in figure 4.

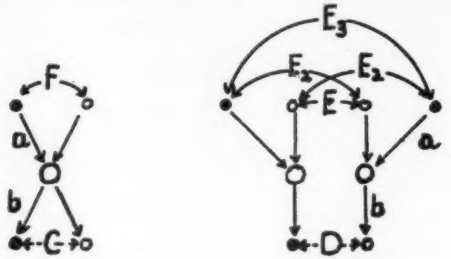

FIGURE 4

$$E = (C/N) + D(N-1)/N \tag{57}$$

$$F = hC + (1-h)D. \tag{58}$$

In cases of cross pollination, the variance of father's mother relative to mother's mother is the same as that of father relative to mother (σ^2) because of the postulated absence of dispersion in the female line. The correlation between ovules from which fathers and mothers (where different) were derived is thus E. The variance of father's father about mother's mother (father and mother different) is $(1+r)\sigma^2$, corresponding to an effective population of $(1+r)N$ if area continuity or $\sqrt{1+r}\,N$ if linear continuity, assuming that N is at least 2. The same is true for father's mother relative to mother's father. The correlation between pollen and ovule produced by these grandparents will be called E_2. The variance of father's father relative to mother's father (father and mother different) is $(1+2r)\sigma^2$, corresponding to an effective population of $(1+2r)N$ if area continuity and $\sqrt{1+2r}\,N$ if linear continuity. The correlation between pollen grains produced by these grandparents will be called E_3.

The spores produced by the Xth ancestral generation, where the parents are different, are drawn from populations with variances $[1+(X-2)r]\sigma^2$, $[1+(X-1)^2r]\sigma^2$ and $[1+Xr]\sigma^2$ according as ovule-ovule, ovule-pollen or pollen-pollen. The effective population numbers are proportional,

$[1+(X-2)r]N$, etc., if area continuity and proportional to the square roots, $\sqrt{1+(X-2)r}\,N$, etc., if linear continuity. Thus if D_X is the correlation between spores from different individuals of this generation,

$$D_X = (E_X + 2E_{X+1} + E_{X+2})/4 \tag{59}$$

$$C = b^2 = (1 + F)/2. \tag{60}$$

With area continuity,

$$\left.\begin{aligned} E &= \frac{C}{N} + \frac{N-1}{4N}(E + 2E_2 + E_3) \\ E_2 &= \frac{C}{(1+r)N} + \frac{(1+r)N-1}{4(1+r)N}(E_2 + 2E_3 + E_4) \\ E_X &= \frac{C}{[1+(X-1)r]N} + \frac{[1+(X-1)r]N-1}{4[1+(X-1)r]N}(E_X + 2E_{X+1} + E_{X+2}) \\ E_K &= 0. \end{aligned}\right\} \tag{61}$$

The equations above (except the last) are obviously satisfied if all E's equal 1, which, as in the preceding cases, is the limiting value approached as the area of reference is made indefinitely large. The attempt to solve for E with finite N_K leads, as in case 3, to an unmanageable series. An approximate solution may be obtained as follows:

Let $$(E_X + 2E_{X+1} + E_{X+2})/4 = E_{X+1} + \Delta_{X+1} \tag{62}$$

$$\begin{aligned} E = \frac{C}{N}\Bigg\{1 &+ \left(\frac{1}{1+r}\right)\left(\frac{N-1}{N}\right) \\ &+ \left(\frac{1}{1+2r}\right)\left(\frac{N-1}{N}\right)\left[\frac{(1+r)N-1}{(1+r)N}\right]\cdots \\ &+ \Delta_2\left(\frac{N-1}{N}\right) + \Delta_3\left(\frac{N-1}{N}\right)\left[\frac{(1+r)N-1}{(1+r)N}\right]\cdots\Bigg\} \end{aligned} \tag{63}$$

As $\Delta_{X+1} = \frac{1}{4}[(E_X - E_{X+1}) - (E_{X+1} - E_{X+2})]$, the Δ's are equal to fourths of the second differences of the E's. They can easily be shown to be negligibly small, and a first approximation may be obtained by ignoring them.

The series that is left in brackets differs from those considered before and may be called $\Sigma t_A'$.

$$E = C\sum t_A'/N \quad \text{approximately} \tag{64}$$

It can easily be found from the equations given above, including the approximation $D = E_2 = (NE - C)/(N-1)$ from (57), the first equation of the set (61), (58) and (60) that

$$C = (1 + rE)/(1 + r) = (1 + F)/2 \tag{65}$$

$$F = [1 + r\ (2E - 1)]/(1 + r) \tag{66}$$

$$\text{Thus}\quad E = \left(\frac{1 + rE}{1 + r}\right)\frac{\sum t_A'}{N} = \frac{\sum t_A'}{N + r(N - \sum t_A')}\quad \text{approximately} \tag{67}$$

The series $\Sigma t_A'$ can be evaluated in the same way as Σt_A (WRIGHT 1943a). If self fertilization occurs only at random in the neighborhood ($r=1$), $\Sigma t_A'$ becomes in fact Σt_A and

$$E = \sum t_A/[2N - \sum t_A]\quad \text{approximately} \tag{68}$$

This is the same formula as that obtained earlier for hermaphrodites with equal dispersion of both parents relative to offspring. However, there is greater differentiation for a given density of population and given dispersion of pollen, since effective N is only half as great if there is no dispersion of ovules as with dispersion of ovules equal to that of pollen.

If $1/r$ is an integer (H), the series $\Sigma t_A'$ can be expressed in terms of Σt_A. Let $N'=rN$ and assume, as before, that this is at least 2. Σt_A below is defined in terms of N' instead of N.

$$\sum t_A' = H\left[\frac{1}{H} + \left(\frac{1}{H+1}\right)\left(1 - \frac{1}{HN'}\right) + \left(\frac{1}{H+2}\right)\left(1 - \frac{1}{HN'}\right)\left[1 - \frac{1}{(H+1)N'}\right] \cdots\right] \tag{69}$$

$$\text{If}\quad r = 1/2,\ H = 2,\quad \sum t_A' = HN'(\sum t_A - 1)/(N' - 1) \tag{70}$$

$$E = 2(\sum t_A - 1)/[3N' - \sum t_A - 2] \tag{71}$$

COMPARISON OF CASES

The effects of different systems of mating on populations with uniform distributions over large areas are compared in tables 1 to 4. Consider first the variance (σ_q^2) of gene frequencies of neighborhoods with populations of effective size 200 (table 1) (excluding in this and all other cases the variance, $(q(1-q)/2N)$, due immediately to accidents of sampling). In an indefinitely extended population of hermaphrodites in which the gametes combine at random in the neighborhood and are dispersed equally along both parental lines, σ_q^2 reaches only 2.45 per cent of its maximum within an area that includes two million individuals and only 4.75 per cent of the maximum if 20 billion are included. Complete exclusion of self fertilization makes no appreciable difference. There is also no appreciable difference if the neighborhoods consist of separate sexes that mate at random to form 100 permanent pairs, assuming equal dispersion of males and females (σ_q^2 for neighborhoods rises to 2.51 per cent of the maximum within an area including one million pairs and to 4.97 per cent of the maximum within an area including ten billion pairs). If each offspring is produced by a separate random mating but otherwise conditions are the same, differentiation of neighborhoods is slightly greater ($\sigma_q^2 = 2.64$ per

TABLE 1

The correlation (E) between gametes of adjacent individuals belonging to neighborhoods of size $N(=200)$, relative to populations of size $N_K(=KN)$, which in turn are portions of a population distributed with uniform density (d) over an indefinitely large area. The effective population of a neighborhood is determined by the density and the variance (σ^2) of parent-offspring distances. $N=4\pi\sigma^2 d$. E also measures the variance, σ_q^2, of gene frequencies of neighborhoods relative to the limiting value $q_K(1-q_K)$ of the population of size N_K. $E=\sigma_q^2/q_K(1-q_K)$.

Case 1 is that of a population of hermaphrodites with various amounts (h) of self fertilization. Case 2 is that of permanent pairs with various amounts (h) of brother-sister mating. Case 3 is that of a separate random mating for each offspring. The results would be the same for $N_m=50$, $N_f=\infty$ as for $N_m=N_f=100$. Equal dispersion in the male and female lines is postulated in cases 1, 2 and 3. Case 4 is that of hermaphrodites with no dispersion in the female line, dispersion of double variance in the male line.

K	N_K	HERMAPHRODITES, N = 200					
		CASE 1 EQUAL DISPERSION OF ♀ AND ♂ GAMETES $\sigma_f^2=\sigma_m^2=\sigma^2$				CASE 4 $\sigma_f^2=0$ $\sigma_m^2=2\sigma^2$	
		h=0	h=.005	h=.500	h=1	h=.005	h=.5025
1	200	0	0	0	0	0	0
10	2×10^3	.0071	.0071	.0094	.0141	.0071	.0128
10^2	2×10^4	.0129	.0130	.0172	.0256	.0130	.0277
10^3	2×10^5	.0187	.0187	.0248	.0368	.0187	.0428
10^4	2×10^6	.0244	.0245	.0323	.0478	.0245	.0577
10^5	2×10^7	.0302	.0302	.0398	.0587	.0302	.0725
10^6	2×10^8	.0359	.0360	.0473	.0695	.0360	.0872
10^7	2×10^9	.0416	.0417	.0548	.0801	.0417	.1018
10^8	2×10^{10}	.0474	.0475	.0622	.0906	.0475	.1162

K	N_K	SEPARATE SEXES $N_m=100$; $N_f=100$				
		CASE 2 PERMANENT PAIRS $\sigma_f^2=\sigma_m^2=\sigma^2$				CASE 3 RANDOM MATING $\sigma_f^2=\sigma_m^2=\sigma^2$
		h=0	h=.01	h=.50	h=1	
1	200	0	0	0	0	0
10	2×10^3	.0071	.0071	.0113	.0280	.0073
10^2	2×10^4	.0130	.0131	.0207	.0505	.0135
10^3	2×10^5	.0189	.0191	.0300	.0722	.0198
10^4	2×10^6	.0249	.0251	.0393	.0933	.0264
10^5	2×10^7	.0309	.0312	.0486	.1140	.0331
10^6	2×10^8	.0370	.0373	.0580	.1341	.0401
10^7	2×10^9	.0432	.0435	.0674	.1538	.0473
10^8	2×10^{10}	.0494	.0497	.0768	.1731	.0547

cent of the maximum in areas including one million of each sex and 5.47 per cent in areas including ten billion of each). If, in a population of monoecious plants, there is no appreciable dispersion of seed but dispersion of pollen of

TABLE 2

The standard deviations $\sigma_{q(i)}$ of gene frequencies of populations of size N_i relative to that of size 2×10^{10} where the effective size of neighborhood is 200. Cases as in table 1

$$\sigma_{q(i)}/\sigma_{q(K)} = \sqrt{(E_K - E_4)/(1 - E_4)}$$

K	N_i	HERMAPHRODITES N = 200					
		CASE 1 EQUAL DISPERSION OF ♀ AND ♂ GAMETES $\sigma_f^2=\sigma_m^2=\sigma^2$				CASE 4 $\sigma_f^2=0$ $\sigma_m^2=2\sigma^2$	
		h = 0	h = .005	h = .500	h = 1	h = .005	h = .5025
1	200	.2176	.2179	.2494	.3011	.2179	.3409
10	2×10^2	.2014	.2017	.2309	.2787	.2017	.3236
10^2	2×10^4	.1868	.1870	.2141	.2584	.1870	.3017
10^3	2×10^5	.1710	.1712	.1959	.2365	.1712	.2770
10^4	2×10^6	.1533	.1535	.1758	.2121	.1535	.2491
10^5	2×10^7	.1332	.1333	.1526	.1843	.1333	.2170
10^6	2×10^8	.1091	.1092	.1250	.1509	.1092	.1782
10^7	2×10^9	.0773	.0774	.0886	.1070	.0774	.1267
10^8	2×10^{10}	0	0	0	0	0	0

K	N_i	SEPARATE SEXES N_f = 100, N_m = 100				
		CASE 2 PERMANENT PAIRS $\sigma_f^2=\sigma_m^2=\sigma^2$				CASE 3 RANDOM MATING $\sigma_f^2=\sigma_m^2=\sigma^2$
		h = 0	h = .01	h = .50	h = 1	
1	200	.2222	.2230	.2772	.4160	.2338
10	2×10^2	.2064	.2071	.2574	.3864	.2185
10^2	2×10^4	.1919	.1926	.2394	.3593	.2043
10^3	2×10^5	.1762	.1768	.2197	.3298	.1885
10^4	2×10^6	.1584	.1590	.1976	.2966	.1705
10^5	2×10^7	.1380	.1385	.1721	.2584	.1493
10^6	2×10^8	.1133	.1137	.1413	.2171	.1233
10^7	2×10^9	.0806	.0809	.1005	.1509	.0882
10^8	2×10^{10}	0	0	0	0	0

twice the variance postulated in the preceding cases (in populations of the same density), the amount of differentiation is practically the same as in the case of hermaphrodites with equal dispersion along both parental lines. This rule applies in random breeding populations, irrespective of density, and applies under linear as well as area continuity. It may be surmised that in such populations the effects with unequal dispersion are substantially the same as if there were equal dispersion to the extent of the average variance along the two parental lines. Returning to the case of neighborhoods of size 200 in a population continuous over an area, it may be added that with dispersion of pollen equal to that in case 1 and no dispersion of seed, σ_q^2 is 5.24 per cent instead of

TABLE 3

The correlation (E) between gametes of adjacent individuals, belonging to neighborhoods of size N = 20, relative to populations of size N_K, which in turn are portions of a population distributed with uniform density over an indefinitely large area. The cases are as in table 1. In case 3 the results would be the same for neighborhoods containing five males and an indefinite number of females as with ten of both.

		HERMAPHRODITES N = 20					
		CASE 1 EQUAL DISPERSION OF ♀ AND ♂ GAMETES $\sigma_f^2 = \sigma_m^2 = \sigma^2$				CASE 4 $\sigma_f^2 = 0$ $\sigma_m^2 = 2\sigma^2$	
K	N_K	h = 0	h = .05	h = .50	h = 1	h = .05	h = .525
1	20	0	0	0	0	0	0
10	2×10^1	.0699	.0716	.0918	.1336	.0716	.1260
10^2	2×10^2	.1269	.1298	.1634	.2297	.1298	.2593
10^3	2×10^3	.1821	.1860	.2304	.3136	.1860	.3799
10^4	2×10^4	.2362	.2409	.2937	.3883	.2409	.4856
10^5	2×10^5	.2890	.2943	.3534	.4548	.2943	.5764
10^6	2×10^6	.3401	.3460	.4094	.5141	.3460	.6535
10^7	2×10^7	.3894	.3956	.4617	.5669	.3956	.7181
10^8	2×10^8	.4366	.4430	.5104	.6140	.4430	.7716

		SEPARATE SEXES $N_f = 10$, $N_m = 10$				
		CASE 2 PERMANENT PAIRS $\sigma_f^2 = \sigma_m^2 = \sigma^2$				CASE 3 RANDOM MATING $\sigma_f^2 = \sigma_m^2 = \sigma^2$
K	N_K	h = 0	h = .10	h = .50	n = 1	
1	20	0	0	0	0	0
10	2×10^1	.0723	.0779	.1124	.2525	.0932
10^2	2×10^2	.1378	.1476	.2062	.4092	.1936
10^3	2×10^3	.2071	.2206	.2980	.5310	.3124
10^4	2×10^4	.2799	.2963	.3871	.6275	.4423
10^5	2×10^5	.3545	.3730	.4715	.7041	.5708
10^6	2×10^6	.4289	.4486	.5496	.7649	.6856
10^7	2×10^7	.5013	.5213	.6203	.8133	.7792
10^8	2×10^8	.5699	.5894	.6829	.8517	.8500

2.45 per cent of the maximum in areas including two million individuals and is 9.82 per cent instead of 4.75 per cent in areas including twenty billion.

A tendency toward self fertilization or toward brother-sister mating somewhat increases the amount of differentiation of neighborhoods. But even 50 per cent self fertilization in the case of hermaphrodites and 50 per cent brother-sister mating in the case of permanent pairs raise σ_q^2 as a percentage of the maximum within a population of 20 billion only from 4.75 per cent to 6.22 per cent in the former and from 4.97 per cent to 7.68 per cent in the latter. The ef-

TABLE 4

The standard deviations of gene frequencies of populations of size N_i relative to that of a population of size 2×10^9, where the effective size of neighborhood is 20. Cases as in table 1.

K	N_i	HERMAPHRODITES N = 20					
		CASE 1 EQUAL DISPERSION OF ♂ AND ♀ GAMETES $\sigma_f^2=\sigma_m^2=\sigma^2$				CASE 4 $\sigma_f^2=0$ $\sigma_m^2=2\sigma^2$	
		h=0	h=.05	h=.50	h=1	h=.05	h=.525
1	20	.6585	.6657	.7144	.7836	.6657	.8784
10	2×10^2	.6279	.6325	.6789	.7447	.6325	.8595
10^2	2×10^3	.5956	.6000	.6440	.7064	.6000	.8317
10^3	2×10^4	.5579	.5620	.6031	.6616	.5620	.7948
10^4	2×10^5	.5123	.5160	.5538	.6075	.5160	.7457
10^5	2×10^6	.4557	.4590	.4927	.5404	.4590	.6789
10^6	2×10^7	.3824	.3852	.4135	.4535	.3852	.5839
10^7	2×10^8	.2781	.2801	.3007	.3298	.2801	.4359
10^8	2×10^9	0	0	0	0	0	0

K	N_i	SEPARATE SEXES $N_f=10$ $N_m=10$				
		CASE 3 PERMANENT PAIRS $\sigma_f^2=\sigma_m^2=\sigma^2$				CASE 4 RANDOM MATING $\sigma_f^2=\sigma_m^2=\sigma^2$
		h=0	h=.10	h=.50	h=1	
1	20	.7549	.7677	.8264	.9229	.9220
10	2×10^2	.7324	.7448	.8017	.8953	.9136
10^2	2×10^3	.7079	.7200	.7749	.8654	.9022
10^3	2×10^4	.6764	.6879	.7404	.8269	.8842
10^4	2×10^5	.6346	.6454	.6947	.7758	.8550
10^5	2×10^6	.5777	.5875	.6324	.7063	.8065
10^6	2×10^7	.4969	.5054	.5440	.6075	.7231
10^7	2×10^8	.3710	.3773	.4061	.4535	.5662
10^8	2×10^9	0	0	0	0	0

fect of self fertilization is naturally much greater in the case of no dispersion of seed but dispersion of pollen of two fold variance. With about 50 per sent self fertilization, σ_q^2 within populations of 20 billion is raised from 4.75 per cent to 11.62 per cent of the maximum.

The differentiation of neighborhoods with 200 individuals that reproduce by exclusive self fertilization is measured by a variance 9.06 per cent of maximum within a population of 20 billion. The case of neighborhoods consisting of 100 brother-sister pairs is the same as for ones of 100 individuals that reproduce by self fertilization. The variance of q reaches 17.31 per cent of maximum within a population of 20 billion. All individuals of course become homozygous in these cases, but neighborhoods become homallelic only to a slight extent, assuming as throughout, no differential selection. Exclusive vegetative mul-

tiplication differs in not leading to homozygosis. The differentiation of neighborhoods is the same as in the above cases, taking q as the frequency of one of two alternative genotypes.

Even the largest of the values of σ_q^2 in table 1 may appear rather small, suggesting that, if the effective size of neighborhoods is as large as 200, there is no important differentiation due to the cumulative effects of accidents of sampling even within enormous areas. However, the square roots of these figures give perhaps a fairer idea of amount of differentiation. Thus values of σ_q of 22 per cent to 24 per cent of the possible maximum, occurring independently at all heterallelic loci, are large enough to give the basis for great diversity among the gene systems of neighborhoods and thus a basis for intergroup selection. But groups of only 200 individuals are rather small, and it is desirable to consider the amount of differentiation among larger groups. Table 2 shows the standard deviation $\sigma_{q(i)}$ for populations of various effective sizes N_i within a comprehensive population of 20 billion, in relation to the maximum standard deviation. For populations consisting of 10 per cent of the total, mean gene frequency varies about one-third as much as it does for neighborhoods. It varies about half as much among groups that include 1 per cent of the total and about two-thirds as much among ones that include 0.1 per cent of the total (that is, populations of 20 million within one of 20 billion). There is therefore significant, if not very great, chance differentiation of large groups, if the effective size of the neighborhood is as large as 200. As noted in the previous paper, there is virtual equivalence to panmixia with regard to chance differentiation where neighborhoods are larger than 1000 (WRIGHT 1943a). All of this discussion, it should be noted, applies only to area continuity. Under linear continuity there is enormously more differentiation.

Tables 3 and 4 present the same cases for $N = 20$ that are presented in tables 1 and 2 for $N = 200$. They show that there is very much greater differentitaion where the neighborhood is small. There is also much more difference in the results of diverse systems of mating. Thus for neighborhoods of 20 random breeding hermaphrodites the variance of q within areas including the equivalent of 100 million neighborhoods is 44.3 per cent of its maximum, for neighborhoods of ten permanent pairs within the same total it is 58.9 per cent of maximum, and for neighborhoods of ten males and ten females, mating at random to produce each offspring, it is 85.0 per cent of maximum, assuming equal dispersion along both lines in all these cases. Table 4 gives the standard deviation of q among populations of various sizes N_i within a constant total of two billion individuals. We note that populations including 1 per cent of this total are strongly differentiated where neighborhoods are as small as 20. With neighborhoods of 20 hermaphrodites with equal dispersion along both lines, the standard deviation of mean gene frequency of populations of 20 million within 2 billion is 38.5 per cent of maximum, for neighborhoods of ten permanent pairs the corresponding figure is 50.5 per cent, and for neighborhoods in which each offspring is the product of a separate random mating among ten males and ten females, the figure is 72.3 per cent.

It was noted in the preceding paper (WRIGHT 1943a) that either reversible mutation or occasional long range dispersion (rate m_1) puts a sharp upper limit on the amount of differentiation among neighborhoods and prevents differentiation of groups larger than about $3/m_1$ neighborhoods. A correction should be made to a statement on p. 131, referred to again in the summary.

"Under exclusive uniparental reproduction, the chance that an individual is derived from the parental population without mutation is $(1-m_1)$ instead of $(1-m_1)^2$. Each term in the series is accordingly to be multiplied by $(1-m_1)^x$ (where X is the rank of the term in question)." This is correct if q refers to the frequency of one of only two alternative genotypes, which are multiplying vegetatively. In reference to gene frequencies of a diploid organism the factor to be applied to terms in Σt is $(1-m_1)^{2x}$, irrespective of system of mating.

It should be added that the occurrence of mutations in an indefinitely extended series of multiple alleles, far from limiting the amount of local differentiation, would greatly increase it. The slow process of diffusion of new alleles from small neighborhoods may not keep up with further mutation and thus each allele may have only a local distribution.

Uniform selection pressure limits differentiation, while local differences in the conditions of selection may enormously increase it, provided that dispersion is sufficiently slow to prevent swamping by panmixia. But even with no differences in the external conditions in different localities, mere chance differentiation at all loci of the sort discussed here may be expected to bring about differences in the direction of selection pressure on individual loci in regions in which different gene systems have been arrived at. The differences in selective trends may be expected to be cumulative, with intergroup selection by means of differential rates of dispersal as a further consequence.

The variability of frequencies of two alleles, due to the cumulative effects of accidents of sampling is thus merely the foundation for much more significant evolutionary processes.

SUMMARY

The properties of large, uniformly distributed populations depend on the system of mating and the effective population number (N) of the random breeding "neighborhoods." It is shown that with density d, of breeding individuals and with standard deviation σ, of the coordinates of location of parents at some phase of their life cycles relative to the corresponding phase for offspring, $N = 2\sqrt{\pi}\sigma d$ if the population has an essentially one dimensional distribution (for example, shore line, river), and $N = 4\pi\sigma^2 d$ if there is continuity over an extensive area. Random mating and equal parental dispersions are assumed here. If there is no dispersion along one parental line (for example, no dispersion of seed) but dispersion measured by variance $2\sigma^2$ along the other line (for example, of pollen), the formulae are the same.

The differentiation of mean gene frequencies among neighborhoods or larger areas, within still larger areas, is investigated in four cases. (1) Populations of hermaphrodites, derived from equally dispersed gametes derived at random

from the neighborhood except for a specified tendency toward self fertilization. (2) Populations consisting of permanent pairs, derived by random mating from the neighborhood, except for a specified tendency toward brother-sister mating, with equal dispersion of the sexes. (3) Populations in which each individual is produced by a separate random mating from the neighborhood. The densities of the populations of the two sexes may differ in this case, but dispersion is assumed to be the same. (4) Populations of hermaphrodites, derived from union of ♀ gametes that are not dispersed to any appreciable extent with ♂ gametes derived at random from the neighborhood, except for a specified tendency toward self fertilization.

If the effective breeding population of neighborhoods is 200, there is a moderate amount of differentiation among large subgroups as well as among neighborhoods, within still larger groups inhabiting an indefinitely large area. There are only slight differences among the four cases. Even 100 per cent self fertilization in case 1 or 100 per cent brother-sister mating in case 2 increases differentiation only to a rather slight extent.

If the effective breeding population of neighborhoods is only 20, there is great differentiation among large subgroups as well as among neighborhoods. The amount of differentiation is considerably greater in case (2) than in case (1) or (4) and considerably greater in case (3) than in case (2).

It is noted that this differentiation, due to cumulative effects of accidents of sampling, may be expected in actual cases to be complicated by the effects of occasional long range dispersal, mutation, and selection, but that in combination with these it gives the foundation for much more significant evolutionary processes than these factors can provide separately.

LITERATURE CITED

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CYTOLOGICAL STUDIES IN CULEX
I. SOMATIC REDUCTION DIVISIONS[1]*

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INTRODUCTION

IN 1917 HOLT found that during the metamorphosis of the larval intestine of the mosquito, *Culex pipiens*, dividing cells with various multiples of the diploid chromosome number, six, were present simultaneously in the epithelial cells. She called these polyploid cells multiple complex cells. HOLT believed that after several divisions these multiple complex cells underwent cytolysis and that the alimentary epithelium of the imago took its origin from small regenerative cells present between the larger multiple complex cells. This view was similar to that of two earlier workers (HURST 1896; THOMPSON 1905) who reported partial or complete histolysis of the intestinal epithelium during metamorphosis.

In a reinvestigation of the origin and fate of the multiple complex cells, BERGER (1938) found evidence that they did not undergo cytolysis but divided one or more times to form the smaller cells of the ileo-colon of the adult. The size reduction was accompanied by a corresponding reduction in chromosome number. He called these divisions somatic reduction divisions. This contribution was unique for several reasons. First, tissues believed by previous observers to undergo partial or complete histolysis were here reported to divide during metamorphosis and contribute to the formation of adult tissues. Second, cells which had attained a high degree of polyploidy had nevertheless retained the ability to divide. Third, a normally occurring process of chromosome reduction in somatic tissue, such as he proposed, was not known to occur elsewhere. However, divisions more or less resembling such true somatic reduction division had previously been recorded. BUSHNELL (1936) reported a "pseudo-reduction" in the metamorphosis of the mid-gut epithelium of the larval bean weevil whereby the large epithelial cells apparently budded off smaller cells which then, together with some basal cytoplasm, separated from the larval cells to form regeneration syncytia. He gave no explanation of the way in which this process of "pseudo-reduction" was accomplished.

STRASBURGER (1907, 1909) and KEMP (1910) were unable to confirm the report of NEMEC (1904) that experimentally induced tetraploid cells in the root tips of Pisum were restored to the diploid condition by a process of somatic

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reduction. Similarly, HANSEMANN (1904), STRASBURGER (1909) and others demonstrated that figures presented as evidence for the occurrence of somatic reduction division in cancerous tissues were abnormal somatic mitoses and therefore not typical reduction division figures. HUGHES-SCHRADER (1927) concluded that the haploid nuclei found among the diploid nuclei of the hermaphroditic gonad of the coccid, *Icerya purchasi*, originated by a reduction division. WINKLER (1910a, 1910b) reported reduction divisions in the hybrid, *Solanum Darwinianum*, whereby the 96 chromosomes of the hybrid are reduced to the 24 in the germ cells. He offered no cytological explanation for the process. Somatic reduction divisions in the multiple complex cells of Culex as suggested by BERGER (1938) involved a separation of previously synapsed homologous or sister chromosomes at early anaphase. At late anaphase chromosomes again united in pairs destined to separate at the ensuing division.

In view of the conflicting opinions regarding the fate of the multiple complex cells, as well as by virtue of the intrinsic significance of a process of somatic reduction division, a detailed study of the cytology of the multiple complex cells was undertaken. It seemed probable that the process of somatic reduction in these multiple complex cells might incorporate phenomena found in mitosis and in meiosis. Therefore, comparative studies of ordinary diploid somatic mitosis and of meiosis as it occurs in spermatogenesis in *Culex pipiens* were used as a basis for interpreting the divisions of the multiple complex cells. The present paper embodies the results of such a study of the somatic reduction divisions of the multiple complex cells. In a subsequent paper mitotic and meiotic divisions in Culex will be described. These descriptions will be accompanied by a discussion and interpretation of somatic reduction, based on the comparative study of the three types of cells. Because of its fundamental significance in the process of chromosome reduction, special attention is given to pairing, particularly at prophase.

Somatic reduction division is intimately linked with somatic pairing. The original observations of STEVENS (1908, 1910) and especially of METZ (1914, 1916) on the close association of homologous chromosomes in the somatic cells of the Diptera have been the starting point for a series of significant contributions regarding pairing. Somatic pairing varies in degree from complete or almost complete association of homologous units throughout the cell cycle (METZ 1916), to regional asynapsis (METZ 1922), to almost complete (METZ, MOSES, and HOPPE 1926) or complete (METZ 1931) non-pairing of somatic or of gonial chromosomes. METZ (1916, 1922) and HOLT (1917) reported somatic pairing in polyploid cells of the Diptera, but neither recorded a closer association of sister than of non-sister elements within the cells. METZ suggested that possibly the equivalence of association applies only to the gross relationship and does not represent the actual interrelations beyond the limit of visibility. In the present study of the multiple complex cells the problem of sister and non-sister pairing has been more fully elucidated.

Variations in the degree of somatic pairing are reflected in differences in the degree of pairing in meiotic cells in the Diptera. MOFFETT (1936) has sum-

marized the variations in meiotic pairing in the Diptera into three types: (1) Meiosis of the normal type with chiasmata in both male and female, as in Culex. (2) Normal meiosis in the female and complete failure of pairing in the male, as in Sciara (METZ, MOSES, and HOPPE 1926). (3) Meiosis apparently normal in the female, and in the male pairing and chiasmata formation in the sex chromosomes but not in the autosomes, as in *Drosophila pseudoobscura* (DARLINGTON 1934). Exception has recently been taken to the existence of chiasmata in the last mentioned case (for example, COOPER 1944). In each type meiosis is apparently normal in the female, and in Culex, a primitive dipteran, meiosis is normal in both male and female. The modified behavior in the males of Sciara and Drosophila must therefore represent conditions specialized beyond those of Culex which, as a primitive type, should offer a basis for the interpretation of anomalous conditions in other Diptera.

The relation between meiotic synapsis and somatic pairing is not well understood. There is a growing tendency to consider meiotic and somatic pairing as different degrees of the same or a similar phenomenon (PAINTER 1933, 1940; KING and BEAMS 1934; SCHRADER 1944), especially since the discovery of somatic pairing in the giant salivary gland nuclei in the Diptera. Much of the confusion regarding the terminology relative to the problem of pairing is no doubt due to the failure to understand completely the interrelations between pairing in meiotic, mitotic, polyploid and polytene cells. The interpretation of the present work is built in part on the assumption that the various types of pairing in Culex represent different degrees of the same or a similar phenomenon. In an ever increasing number of cases among the Diptera (for example, STEVENS 1910; METZ 1914; METZ and NONIDEZ 1921, 1923) pairing in prepa-

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EXPLANATION OF FIGURES

FIGURES 1–11.—Photomicrographs of first-division multiple complex prophases from the ileum of *Culex pipiens*. ×1250. Aceto-orcein smear preparations.

FIGURES 1–2.—Early prophase showing relational coiling and pairing of homologous threads (*cf.* arrows).

FIGURE 3.—Early prophase with three pairs of relationally coiled homologous sister-groups, showing characteristic flared regions.

FIGURE 4.—Mid-prophase. Arrow indicates flared region of one of the chromosome pairs, with a row of chromatic granules across the diameter of the flare.

FIGURE 5.—Mid-prophase bundles, each consisting of paired homologous groups. Arrow points to flared region.

FIGURE 6.—Two mid-prophase bundles, each consisting of a pair of relationally coiled homologous sister-groups. Pairing of sister chromosomes in two's is shown in the flared regions.

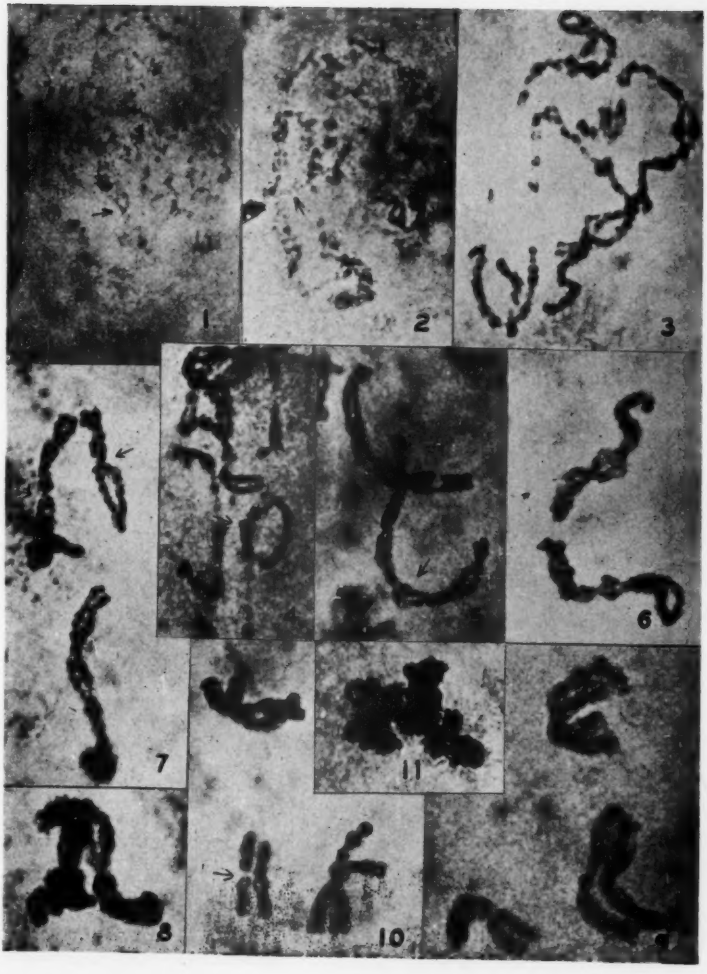
FIGURE 7.—Mid-prophase with three bundles, each consisting of a pair of homologous sister-groups. Arrows indicate apparently fused SA-regions.

FIGURE 8.—Late prophase showing three pairs of homologous bundles held together at a common SA-region. Flared regions are visible below the SA-regions.

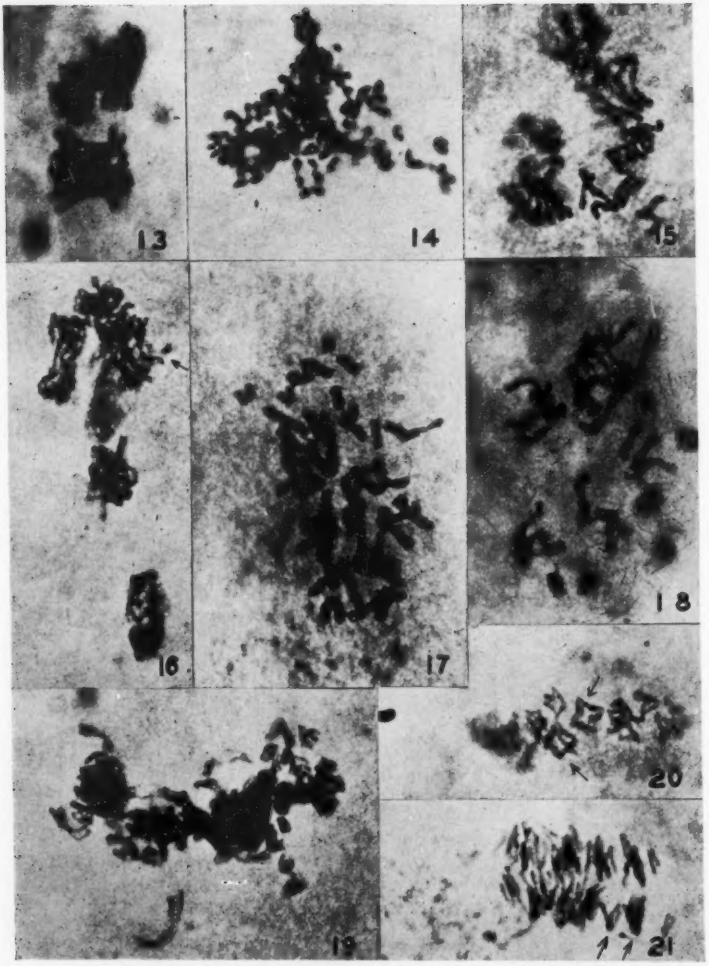
FIGURE 9.—Late prophase showing transition from the three- to the six-bundle stage. Lower left bundle shows fused SA-region.

FIGURE 10.—Late prophase, six-bundle stage. Homologous groups are separate but closely paired. Arrow indicates the position of undivided SA-regions of sister chromosomes.

FIGURE 11.—Late prophase, six-bundle stage.



FIGURES 1–11



FIGURES 13–21

ration for meiotic synapsis is reported to occur at the last premeiotic anaphase or telophase. It is not altogether clear whether this premeiotic association is synapsis, or whether it predisposes homologous threads for synapsis. In a recent paper SMITH (1942) has extended premeiotic pairing to all Diptera and to many other organisms. COOPER (1944) takes issue with SMITH'S assumption of singleness of anaphase chromosomes to account for the pairing. Anaphase pairing and singleness of anaphase chromosomes are among the critical issues in the present work. Culex is especially well adapted for use in studying problems of pairing because of the varied cell types it contains, ordinary somatic, meiotic, multiple complex and giant salivary gland cells, and because of the persistence of pairing in varying degrees throughout the cell cycles. The first three cell types are included as a part of the present investigation. A study of the salivary gland chromosomes of Culex is in progress.

The following terminology is employed in the present paper. The term "synapsis" is restricted to the gene to gene or chromomere to chromomere apposition of homologous chromosomes in meiosis (SCHRADER 1944). To avoid ambiguity in the use of the term "somatic pairing" in the highly polyploid multiple complex cells, a distinction is drawn between two kinds of somatic pairing: (1) "homologue pairing," the pairing of homologous units, and (2) "sister pairing," the association of sister chromosomes within the individual homologous units in the multiple complex cells. "Somatic synapsis" is used for the phenomenon whereby threads that had undergone active somatic pairing at the previous anaphase enter into a more intimate union in the somatic reduction process. The term was first used by PAINTER (1934) to describe the pairing in the salivary gland nuclei of Drosophila.

EXPLANATION OF FIGURES

FIGURES 13–21.—Photomicrographs of first-division multiple complex divisions from the ileum of *Culex pipiens*. ×1250. Aceto-orcein smear preparations.

FIGURE 13.—Prometaphase bundles with chromosomes showing undivided SA-regions and unseparated arms. 16-ploid.

FIGURE 14.—32-ploid metaphase. SA-regions of chromatids are undivided.

FIGURE 15.—16-ploid prometaphase. The chromosomes lie in six groups of eight sister chromosomes each. Homologous groups lie near each other.

FIGURE 16.—16-ploid prometaphase. Relational coiling in two's and pairs of two's is seen in the group of sister chromosomes at the upper right (*cf.* arrow).

FIGURE 17.—Metaphase and early anaphase of a 16-ploid cell. SA-regions and chromatid arms are separate. Note the clear somatic coil.

FIGURE 18.—Prometaphase or metaphase of an octoploid cell. Each chromosome consists of two paired chromatids with SA-regions and arms completely separated.

FIGURE 19.—Anaphase. The small group of chromosomes at the right belong to one cell, the remainder of the chromosomes belong to another cell. Chromosomes at the left and middle show anaphase separation beginning simultaneously with the falling apart of the prometaphase bundles.

FIGURE 20.—Early anaphase of an octoploid cell showing pairing of homologous (or sister) anaphase chromosomes (*cf.* arrows).

FIGURE 21.—Anaphase showing somatic pairing of homologous chromosomes en route to the poles (*cf.* arrows).

MATERIALS AND METHODS

Egg rafts and larvae of *Culex pipiens* were obtained from the NEW YORK BOTANICAL GARDEN, the FORDHAM UNIVERSITY campus, and from Woods Hole, Massachusetts. Culture methods consisted essentially of raising larvae in dishes of tap water to which was added as food a water suspension of a mixture of equal parts of powdered milk, powdered yeast, and dried blood. The water and food were renewed daily. Individual egg rafts were reared separately to facilitate species identification. The adult mosquitoes were bred in cages and were given an occasional meal of bird blood.

The methods of preparation used included aceto-orcein smears, Feulgen-stained total mounts, and paraffin sections. In all cases dissections were made in isotonic Ringer's solution. The aceto-orcein smear technique was used for determining the fine cytological details of the multiple complex cells of the ileum and rectum before, during, and after the metamorphosis of the hind-gut, for diploid somatic divisions in the mid-gut, brain, nerve ganglia and limb buds of the larvae and pupae, and for the testes of second and third instar larvae and pupae. Frequently the tissues were observed under the microscope during the staining process, before being smeared, in order to determine the proper spatial relationships of the cells. Also, in this way details of structures, especially relic coiling, sometimes lost after smearing were seen.

Feulgen-stained total mounts of larval and pupal hind-guts and of gonads were useful in a reinvestigation of the progress of metamorphosis and for determining the normal disposition and structure of the multiple complex cells and the germ cells. This method was an indispensable supplement for the smear preparations. In the sectioned material both Bouin's and Flemming's strong solution gave good fixation. In some instances the hind-gut and the testes were dissected from the mosquitoes before fixation, to facilitate the penetration of the fixative. In other cases only the segments of the abdomen which contained the gonads and the hind-gut were removed and fixed. Sections were cut at six, eight, and ten micra and were stained with Heidenhain's iron-alum-haematoxylin, Orange G being used as a counterstain. The photomicrographs, with one exception, were taken from the aceto-orcein preparations.

OBSERVATIONS

The metamorphosis of the hind-gut of *Culex pipiens*, described by BERGER (1938), was reinvestigated as a basis for the interpretation of the multiple complex cells. The findings were in accord with those reported by BERGER. The hind-gut at the beginning of metamorphosis consists of three main regions —the ileum, colon, and rectum. During metamorphosis the colon disintegrates, while the cells of the ileum and rectum divide to form the ileo-colon of the adult. The dividing cells contain the multiple complexes. They arise by a process of repeated chromosomal reduplication during the long resting stage preceding metamorphosis. This resting stage extends throughout the nine to 14 days of larval life and the first six or seven hours of pupal life. No cell

divisions occur in the ileum during this time, the cells undergoing a progressive increase in size but not in number. This increase in cell size is accompanied by an increase in chromosome number from the diploid number, 6, to 12, 24, 48, 96 and rarely 192. Not all the cells reach the same degree of polyploidy by the end of the growth period when they come into division.

On the suggestion of DR. BERGER, a renewed search for evidence of endomitosis in the cells of the developing ileum was undertaken. A study of Feulgen-stained total mounts and aceto-orcein smears of larval ilea from the time of hatching to the beginning of metamorphosis confirmed BERGER'S conclusion that endomitosis as described by GEITLER (1939) or PAINTER and REINDORP (1939) does not occur. Before metamorphosis the cells of the ileum and of the colon are relatively few in number, large, and variable in size. During metamorphosis these cells undergo one or more somatic reduction divisions to form the larger number of smaller, more regular cells of the ileo-colon. Since these newly formed ileo-colon cells do not divide, there is only indirect evidence for the degree of polyploidy of the cells. It is estimated that the cells are probably tetraploid or octoploid. The mechanism by which these cells with reduced chromosome complexes are formed from highly polyploid multiple complexes is described below.

In the course of metamorphosis the multiple complex cells of the ileum undergo one, two, or more divisions. These divisions were found to be of two types—named, respectively, first-division and later-divisions. A first-division is the initial division which any multiple complex cell undergoes. A later-division is any division other than the first. There may be one or more such later-divisions, depending on the degree of polyploidy at the beginning of metamorphosis of the ileum. All divisions have the usual prophase, metaphase, anaphase, and telophase stages, and two succeeding divisions are separated by short interphases. In the descriptions which follow, succeeding stages of the first- and later-divisions are treated in turn. In each case phenomena of special interest are described in greater detail.

First-division prophase

At the beginning of the metamorphosis of the hind-gut when the nuclei come out of resting stage, the chromatin becomes visible as irregular, slightly chromatic masses arranged more or less in linear order (fig. 1). Imperceptibly this condition changes so that the chromatic material is seen to be in the form of paired, apparently single chromonemata (fig. 2), each of which is later seen to consist of from two to 32 sister threads. There is no evidence for a synapsis of threads in the first-division prophase of the multiple complex cells of Culex. At a very early stage the apparently single chromonemata are seen to be relationally twisted about each other. This relational coiling in earliest prophase is evidence that the multiple sister threads of which each such chromonema is constituted have not previously been separated by endoprophase, endometaphase, and endoanaphase stages as they arose by repeated reproduction. Each cell contains three groups of chromosomes, corresponding to the haploid num-

ber for Culex. This point becomes evident from a study of progressive stages in early prophase (fig. 2, 3). The apparently single chromonemata twisted about one another are homologous units, and since each of these homologous units is later seen to be multiple, the term "sister-group" will be used to designate the combined sister chromosomes comprising each unit. The composite entity resulting from the close association of two homologous sister-groups, or of the members of one such group, will indiscriminately be called a "bundle." The close association of homologous sister-groups is retained until mid- or late prophase, so that at no time prior to that are six groups of chromosomes seen. In early prophase, then, there are three long slender bundles each consisting of two relationally coiled sister-groups (fig. 3). Each sister-group is made up of several sister chromosomes which were formed by the reduplication of threads in the resting stage. Since sister chromosomes come out of resting stage already closely associated, they do not undergo an active pairing. Findings here are in agreement with later views of BERGER (1941) that chromosomes come into prophase already paired. The three long bundles shorten, thicken, and become more chromatic during prophase (fig. 3, 4, 5). As this happens, the relational coiling between the homologous groups of each bundle is gradually lost (fig. 5 to 9), until at late prophase the three pairs of homologous bundles fall apart to form six bundles of sister chromosomes (fig. 10, 11). The six-group stage, therefore, follows the three-group stage. Each of the six groups of chromosomes then falls apart into its constituent prometaphase chromosomes. This process is accompanied by the loss of relational coiling.

Since the prophase of the multiple complex first-division is especially significant, some of the more important points of this stage will be discussed in greater detail.

A. Prophase pairing

New information regarding pairing in the Diptera has been derived from a study of salivary gland chromosomes. Prescinding from the disputed question of whether or not these structures are bundles of chromonemata, all cytologists agree that they consist of two greatly enlarged homologous elements, more or less intimately fused to form a single structure in which the ultimate details of organization are not unequivocally visible. In the dividing multiple complex cells no such fusion occurs. Instead, the disposition of the homologous and sister chromosomes remains visible throughout prophase. This fact makes the mosquito multiple complex cells especially valuable as a factor in determining the kinds and nature of pairing in somatic divisions. The following types of somatic pairing are distinguishable in the prophase of the dividing multiple complex cells: (1) homologue pairing, (2) sister pairing, (3) somatic synapsis. This last type will be considered in the description of the later-division prophases.

1. *Homologue pairing.* Homologue pairing, as previously defined, is either the pairing of homologous chromosomes or of homologous groups of chromosomes. It is the somatic pairing established by METZ (1916) as characteristic of the Diptera. In the mosquito it exists in all somatic divisions, whether diploid or

multiple complex divisions. In diploid prophases it consists of the intimate association of the two members of a pair of homologous chromosomes. Frequently the pairing is so close that the individual homologues are distinguished with difficulty. In the multiple complex prophases homologue pairing exists between homologous groups of chromosomes. This association is more or less intimate, depending on the stage of prophase under consideration (fig. 3, 5, 6). The close association may involve relational half twists, apparently fused regions, and asynaptic regions where the homologues lie parallel but not in actual contact (fig. 5). The relational half twists probably arose at the last preceding somatic mitosis when, in the mosquito, homologous units undergo active pairing. SMITH (1942) has extended this idea and considers active pairing to be a common property of dipteran chromosomes at every anaphase.

The pairing of homologous groups shows an interesting variation in regions which will be designated as "flared regions." For the present, references to figures 3 and 4 will show that the flared region is one in which the chromosomes of a prophase bundle tend to spread out in a specific region of the bundle, giving the appearance of a repulsion both within and between homologous groups in early prophase (fig. 3). The two groups may either be distinct from one another (fig. 5, 7), or they may appear as a single large flare (fig. 4, 6).

The relational coiling between homologous groups has practically or entirely disappeared by late prophase (fig. 8 to 11) resulting in six bundles of sister chromosomes. At times homologous bundles are held in contact at their SA-regions after the relational coils have been undone (fig. 8, 9). Homologue pairing persists even after actual contact between homologous groups is lost. Throughout late prophase (fig. 10, 11), and into prometaphase (fig. 13, 15), homologous bundles are more closely associated than non-homologous ones.

2. *Sister pairing.* Sister pairing is the association of sister chromosomes within each sister-group in multiple complex cells. The pairing relationships of such sister chromosomes may be determined either by studying the grouping of threads in the flared regions of prophase bundles, or by an examination of the condition of the sister chromosomes when the bundles begin to fall apart at prometaphase. Sister chromosomes appear to be paired in two's. The threads most intimately paired in any particular prophase bundles are the chromatids, which may be more or less closely paired, depending, apparently, on the time of the division of the SA-regions. Any two threads which are destined to go to opposite poles at the ensuing anaphase are designated as chromatids. A pair of chromatids constitute a chromosome. Two such chromosomes are relationally coiled to form a pair of sister chromosomes (fig. 16). The orderly arrangement of the chromosomes in a bundle in most of the late prophases and their orderly separation at prometaphase lend support to the idea that pairs of sister chromosomes are associated in a regular order with other such pairs of sister chromosomes. While homologue pairing results from an active coming together of homologous chromosomes at anaphase, sister pairing is not an active process. Instead, it results from the reduplication of threads in the resting cell without a separation of the reproduced elements. This distinction between homologue pairing and sister pairing is reflected in a

variation in the time of the division of the SA-regions. This will be discussed in Part II of this study.

Relational coiling between pairs of sister chromosomes is undone as the chromosomes fall apart at prometaphase. Sister pairing as such, however, is not completely lost until anaphase, for the members of each particular sister-group tend to remain more closely associated than non-sisters.

B. Coiling

The following types of coils are found in first prophase chromosome bundles: (1) somatic coils, (2) relational coils between chromatids, between sister chromosomes, and between pairs of sister chromosomes, (3) relational coils between homologous groups.

1. *Somatic coil.* While the somatic coil may occasionally be seen in early (fig. 2) and late prophase (fig. 11) of first-division, it is most clearly visible

FIGURE 12.—Diagrammatic view of the flared region seen in the first-division multiple complex prophase bundle at the top in figure 6. Four of the eight sister chromosomes constituting each sister-group are shown in the diagram. Relational coiling of homologous groups is visible at the ends of the flared region. Sister chromosomes are paired in two's. Chromomere-like bodies are not SA-regions.

when the bundles begin to fall apart to form prometaphase groups (fig. 16, 17, 18). The gyres are more or less distinct, depending on the particular type of cell in question.

2. *Relational coils between chromatids and between sister chromosomes.* Figure 19, a prometaphase stage of a multiple complex division, shows the relational coils between sister chromatids. Such coiling is especially clear in chromosomes whose SA-regions have divided before prometaphase. Figure 16 shows how two sister chromosomes may be relationally coiled. Sister threads are not evenly spaced in prophase bundles. As noted above, those sister threads which will separate at anaphase are most closely associated. The consistent orderly arrangement of chromosomes in late prophase and prometaphase bundles (fig. 11, 16) makes it appear highly probable that there is a regular arrangement of sister chromosomes in pairs, in pairs of pairs, and in pairs of such pairs, depending on the size of the bundle.

The flared region of the prophase bundles affords further evidence of relational coiling between sister chromosomes. Figure 12 is a diagrammatic view of the flared region in the chromosome group at the top in figure 6. It is presumably 16-ploid, containing eight sister threads in each homologous sister-group in the diagram. The two flared regions here appear as one. In this diagram (1) chromatids are not indicated; (2) sister chromosomes are relationally coiled and close together; (3) a pair of such sister chromosomes relationally

coils about a similar pair; together they constitute four chromosomes forming one sister-group; (4) at the ends of the flared region one sister-group is relationally coiled with its homologue forming one of the three prophase bundles previously described.

3. *Relational coils between homologous bundles.* From the earliest stages at which chromosomes become visible in the first-division prophases, homologous units are seen to be relationally twisted about each other (fig. 1, 2). The number of relational coils decreases as prophase progresses, until at late prophase homologous groups of chromosomes are separate. Relational coiling between homologous units and that between sister chromosomes are apparently similar in nature. The fact that coiling between homologous units appears subsequent to active somatic pairing, while that between sister chromosomes, which are paired as a result of reduplication, appears independent of such active pairing, indicates that relational coiling and its causes are independent of pairing mechanisms.

C. Flared regions of the bundles

Attention was first drawn to the flared regions in the chromosomes by the consistent appearance of a loop-like structure at the mid-region of each of the three pairs of somatically paired polarized homologous chromosomes in mid-prophase of spermatogonial divisions in Culex (Part II of this study). On either side of the spread region the homologues were so closely intertwined that they appeared as one. The observation that flared regions occurred in each of the three pairs of chromosomes at corresponding positions gave the phenomenon added interest. Subsequently it was noted that similar flared regions were present in the mid-region of all multiple complex bundles, adjacent to the SA-regions (fig. 8–10), from early to late prophase when they are lost. Frequently a row of chromatic granules may be seen extending across the widest diameter of the flare (fig. 3, 4, 6, 12). The significance of these has not been established, although possibly they represent minute heteropycnotic regions.

The flared regions have proved to be especially valuable in establishing the sequence of pairing. This is evident from figure 12 which, as mentioned above, represents diagrammatically the pairing of homologous, of sister, and of pairs of paired sister chromosomes.

D. Degree of polyploidy

The discovery of the flared regions in prophase bundles (fig. 3–7) has provided a new method of determining the degree of polyploidy. Repeated observations indicate that in early prophase each optically single thread represents a chromosome. In late prophase the coiling and shortening of the threads tend to conceal the actual number. Preliminary studies show that flared regions are present also in the giant salivary gland chromosomes of Culex. Comparison of these with the dividing multiple complex cells will probably provide evidence on the structure of the giant salivary gland chromosomes in Culex.

E. SA-regions

The SA-regions in the chromosomes of Culex are approximately median in two of the chromosome pairs and slightly sub-median in the third. Whenever

the SA-region can be identified in the multiple complex first-division bundles, it is seen as an achromatic, apparently structureless, region adjacent to the flared region. Bundles such as those represented in figure 7 (upper left bundle) and in figures 8 and 9 (lower left bundle) seem to have a common constriction uniting homologous groups of chromosomes. If such constricted regions connecting homologues are rightly interpreted as undivided spindle attachment regions, a fusion of such regions must have occurred previously. This is a necessary assumption since homologous chromosomes had undergone active pairing at the last anaphase preceding multiple complex formation. The undivided SA-regions between sister chromosomes are more easily explained. Presumably they do not result from a fusion but from delayed division of the SA-region following reduplication of threads in the resting stage.

The sequence of division of the SA-regions is revealed by a study of successive stages of prophase. SA-regions uniting homologous groups are the first to divide during prophase. This division leaves each of the two sister-groups intact (fig. 9–11), with undivided SA-regions. Division of these latter regions connecting pairs of sister chromosomes occurs next. It affects all except the spindle attachment regions which unite sister chromatids. Lastly, the SA-regions uniting chromatids divide. This division may occur before or after prophase bundles fall apart at prometaphase. It is proposed that variations in the types of prometaphase and metaphase chromosomes, as described in the next section, are correlated with the time of division of the SA-regions.

First-division prometaphase

While late prophase bundles closely resemble each other superficially, differences in the condition of the prophase chromosomes constituting the bundles

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EXPLANATION OF FIGURES

FIGURES 22–31.—Photomicrographs of somatic reduction divisions in the multiple complex cells of the ileum of *Culex pipiens* (except figure 24 which is of *C. territans*). ×1250. Figures 22–30 are taken from aceto-orcein smears, figure 31 from a section of the ileum fixed in Bouin's fluid and stained with iron-alum-haematoxylin.

FIGURE 22.—First-division multiple complex telophase. Arrow points to region where doubleness of threads is visible.

FIGURE 23.—Interphase between first- and later-divisions. Note the paired threads in the region indicated by the arrow.

FIGURE 24.—Early later-division prophase of *Culex territans*. The chromosomes are in three groups of homologous units. Note the polarization of threads and remnants of relic coils.

FIGURE 25.—Mid-prophase of a later-division cell. Note polarization. Later-division chromatids are visible (*cf.* arrow).

FIGURE 26.—Late prophase of a later-division cell showing three distinct groups of homologous threads. Each polarized chromosome consists of two closely synapsed homologous chromosomes.

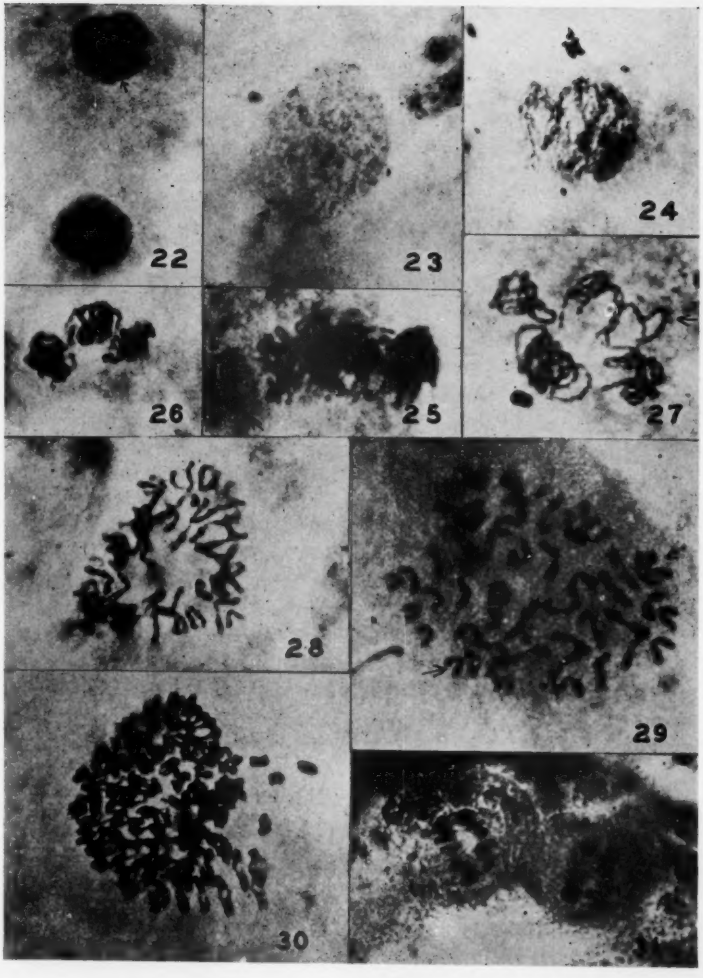
FIGURE 27.—Two daughter later-division prophases. The arrow indicates the position of the SA-region in one of the pairs of synapsed chromosomes. Note the absence of flared regions.

FIGURE 28.—Later-division prometaphase.

FIGURE 29.—Later-division metaphase and very early anaphase. Note the tendency for chromosomes to be associated in two's, with their SA-regions close together (*cf.* arrows).

FIGURE 30.—Later-division early anaphase.

FIGURE 31.—Later-division anaphase showing two daughter cells each with chromosomes going to the poles in groups. Section cut at eight micra.



FIGURES 22–31

become evident as the bundles fall apart to form the prometaphase groups. It is possible to separate prometaphase chromosomes into types which form a more or less continuous series ranging from a condition in which the SA-regions are undivided and chromatid arms are partially or completely in contact (fig. 13, 15, 16) to one in which both SA-regions and chromatid arms are completely separated and lying parallel to each other (fig. 18). The paired, parallel-arranged chromatids in the latter case might well be mistaken for chromosomes which would themselves undergo separation of chromatids at the ensuing anaphase were it not for the fact that their subsequent history clearly shows that the members of each such pair go to opposite poles at anaphase. By definition, therefore, they are chromatids. Half chromatids are not visible in Culex. (For a complete description of prometaphase types cf. GRELL 1945.)

First-division metaphase

Differences in the prometaphase condition of the chromosomes are reflected in the appearance of the metaphase chromosomes. One type of prometaphase chromosomes may come out of the bundles and onto the metaphase plate with SA-regions still undivided and chromatid arms well contracted and in contact (fig. 14). In this case the SA-region divides at or after metaphase orientation. If the chromatid arms but not the SA-regions are divided at prometaphase, the spindle attachment regions seem to divide simultaneously with the alignment of the chromosomes on the metaphase plate. This results in a condition such as that represented in figure 17. The chromatids are closely paired and parallel, the somatic coil is exceptionally clear, and the entire chromosomes tend to be less contracted than in the first mentioned case. Chromosomes which have their SA-regions divided before prometaphase are unique in their subsequent behavior. If the term metaphase signifies the congression of chromosomes onto a metaphase plate, these chromosomes can scarcely be said to undergo a metaphase stage. Anaphase separation of chromatids appears to take place before the late prophase bundles have fallen apart (fig. 19).

First-division anaphase

If the commonly accepted definition of anaphase, the separation of two chromatids of each chromosome at the SA-region and the initiation of their poleward movement, is applied to the multiple complex divisions, it is difficult to determine the exact time when anaphase begins. In two types of first-division chromosomes, at least, the SA-regions and chromatids are already spatially separated before or at metaphase, before anaphase separation actually begins. Possibly some force similar to somatic pairing is effective in keeping chromatids thus paired in a perfectly parallel position, without adhering, and yet without undergoing immediate separation. In early anaphase of first-division (fig. 20, 21) the chromosomes frequently present an irregular, scattered appearance, apparently beginning their movement to the poles in small groups. Sister and homologous chromosomes are no longer distinguishable from each other after anaphase separation begins. Any chromosomes, therefore, which were previously associated in prophase, whether sisters or homologues, will subsequently

be designated as homologues. Active somatic pairing begins with, or shortly after, chromatid separation and continues as the chromosomes move to the poles. This pairing consists of the approximation of homologous chromosomes in two's or in groups of such two's (fig. 20, 21). This is seen to best advantage in anaphase figures while they are staining, before being smeared, and in sections (fig. 31). In smeared preparations the anaphase groups are frequently slightly displaced with reference to each other.

First-division telophase

As the chromatin threads become less chromatic in telophase, it is possible to see that they are paired (fig. 22). Differences between the subsequent prophase behavior of these paired threads and the chromosomes in diploid prophases seem to indicate that the paired elements in these multiple complex telophases are actually first-division anaphase chromosomes and not chromatids. This is an important distinction in the light of somatic reduction.

Interphase

The great similarity between the latest recognizable telophase of first-division and the earliest distinguishable prophase of the succeeding division makes it seem probable that the interphase between two successive multiple complex divisions is very short. No typical resting nuclei are found during the seven or eight hours of metamorphosis. Interphases are few in comparison with dividing cells, indicating a short interphase. In some instances paired threads are in evidence (fig. 23).

Introductory remarks for later-divisions

The term later-division is used to refer to any division of the multiple complex cells other than the first-division. There may be one or several such later-divisions. The mechanism of division for all later-divisions appears to be the same. Chromosome number alone is no criterion for judging the number of divisions a particular cell has undergone, for at any one time during metamorphosis the ileum may contain cells representing the various multiples of chromosomes from the diploid to the 64-ploid condition. Indirect evidence for the number of divisions a cell has undergone is obtained by comparing adjacent cells.

Somatic reduction becomes effective in the later-divisions. Unlike the resting stage preceding ordinary mitotic division, in which chromosome reduplication occurs, evidence shows that *chromosome reduplication does not occur in the interphase between divisions of the multiple complex cells.* Instead, in interphase or very early later-division prophase there is a synapsis of homologous chromosomes which had undergone somatic pairing at the preceding anaphase. Actual numerical reduction is, then, accomplished as these threads undergo synapsis instead of reduplication.

Later-division stages

In general appearance later- and first-division prophases are extremely different (compare fig. 1–11 with fig. 24–27). In later-division the chromosomes

are polarized; they do not unite to form bundles; they are not noticeably relationally coiled about each other; they contain a marked relic coil; the flared regions, if present, are imperceptible. These characters which distinguish a later- from a first-division will be taken up in the following description of the phenomena accompanying later-division.

A. Polarization

The polarization of later-division prophase appears to be carried over from the previous telophase through the short interphase. Unlike first-division, threads are polarized from earliest prophase until prometaphase (fig. 24–28).

B. Pairing

While pairing of chromosomes into bundles of homologues as seen in first-division prophase (fig. 3) does not occur (fig. 24), later-division homologous chromosomes frequently tend to undergo a secondary grouping into three loosely arranged masses of homologues (fig. 24, 26). Each later-division chromosome consists of two synapsed threads. The chromosomes loosely approximate each other but do not lie in intimate contact, nor are they noticeably relationally coiled about each other. In well smeared preparations it is possible to see that the apparently single later-division early prophase threads are double. This doubleness becomes evident when at intervals the two closely synapsed units comprising the apparently single threads spread apart slightly. The synapsis is less intimate than in meiotic prophase. It is impossible to determine with certainty when synapsis of later-division multiple complex chromosomes occurs, but it seems logical to suppose that it occurs at late interphase or early prophase when the chromonemata attain greatest despiralization. The pairing which begins at the previous anaphase and becomes more pronounced at telophase predisposes the paired homologues for the intimate association of early later-division prophase. This association in two's of homologous chromosomes which underwent initial somatic pairing at the previous anaphase, and which are destined to separate subsequently at anaphase, is here referred to as "somatic synapsis." The two threads which synapse are called "later-division chromatids," since they are destined to separate at anaphase. In origin, however, they are either homologous or sister chromosomes of the previous anaphase. Somatic pairing at anaphase does not always predispose homologues for subsequent synapsis. In diploid mitoses in Culex there is somatic pairing but no succeeding synapsis. The difference is probably correlated with the presence of a normal resting stage and reduplication of threads in diploid mitoses and a short interphase without reduplication of threads in multiple complex interphases.

C. Coiling

The fine condition of the closely synapsed later-division chromatids at early prophase and the presence of a rather pronounced somatic coil in later stages makes it impossible to determine whether the chromatids are relationally coiled. Aside from this, later-division prophase is conspicuous for the absence of relational coiling between polarized chromosomes. That the tangling of

threads in early later-division prophase (fig. 24) is not relational coiling becomes evident by comparison with diploid prophases (Part II of this study) and with first-division prophases (fig. 2, 3), in which relational coiling is clearly evident. The difference may be due to the absence of a reduplication of threads between first- and later-division and its presence in the resting cells preceding diploid and first-division multiple complex mitoses. A loose waviness of early prophase threads in later-division prophase suggests a relic coil (fig. 24).

D. Flared regions

Correlated with the absence of close association of threads except between later-division chromatids, there is an absence of a well defined flared region described earlier for first-division prophase bundles.

E. SA-regions

The SA-regions of the synapsed later-division chromatids are in very close contact if not actually fused. They appear as an optically single achromatic region. Separation of these fused SA-regions occurs after the orientation of the chromosomes on the metaphase plate (fig. 29, 30).

It is interesting to note that in later-division metaphase, as in ordinary diploid and in spermatogonial metaphases, the SA-regions of pairs of homologous chromosomes tend to be attracted to each other and that homologous chromosomes of a group tend to lie near each other (fig. 29, cf. arrows). In its fully contracted state the metaphase chromosome has a constricted SA-region, smooth outlined arms, and often no visible split between the chromatid arms. While some chromosomes are still in the metaphase condition, others of the same cell may begin anaphase separation (fig. 29, 30).

Later-division anaphase affects the separation of somatically synapsed homologous chromosomes. Somatic pairing of separating anaphase chromosomes takes place as in anaphase of first-division. This is seen in figure 31 in which two daughter cells are undergoing anaphase separation. Homologous chromosomes have paired and are moving to the poles in groups. Telophase follows as described for the first-division multiple complex cells.

The interpretation of the mechanism of somatic reduction division in the multiple complex cells of *Culex pipiens* and the significance of pairing and other related phenomena are in great part the result of a comparison of these multiple complex cells with meiotic and ordinary diploid somatic divisions in Culex. Data on these last named divisions and a general discussion are to be presented in a subsequent paper.

SUMMARY

Multiple chromosome complexes (4n to 64n) in Culex arise by repeated reproduction of chromosomes in the resting nucleus, not by endomitosis as defined by GEITLER.

At metamorphosis multiple complex cells undergo two kinds of divisions, "first-divisions" and "later-divisions," which reduce the number of chromosomes.

Repeated reduplication in the protracted resting stage preceding first-divi-

sion results in three long slender bundles of prophase chromosomes, each consisting of two relationally coiled homologous groups of sister chromosomes. Sisters are paired in two's, in pairs of two's, and in pairs of such pairs.

The somatic pairing of homologous bundles results from the coming together of homologous chromosomes at the preceding anaphase; that of sisters is the result of reduplication without separation.

Each first-division bundle contains a flared region in which the chromosomes appear to repel each other. These regions afford evidence of the degree of polyploidy, and of the sequence of pairing and relational coiling.

Relational coiling is independent of the pairing mechanism and is lost during first-division prophase.

Each of the three prophase bundles has a single SA-region resulting from the delay in the division of sister SA-regions and the fusion of homologous SA-regions.

Progressive loss of relational coiling and successive division of the SA-region gives rise to separate prometaphase chromosomes. Final division of the SA-region may occur before metaphase.

First- and later-divisions are separated by a short interphase during which no reduplication of chromosomes occurs. Instead, chromosomes paired at anaphase undergo a closer association during interphase (somatic synapsis). The somatically synapsed chromosomes separate and go to opposite poles at later-division anaphase. The substitution of somatic synapsis for chromosome reduplication in the resting stage constitutes the essence of somatic reduction.

Later-division prophases differ from first-division prophases as follows: the chromosomes are polarized; they do not form bundles; they do not contain a conspicuous flared region; and they are not relationally coiled. These differences appear to be correlated with the presence or absence of chromosome reduplication in the resting stage preceding division.

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CYTOLOGICAL STUDIES IN CULEX
II. DIPLOID AND MEIOTIC DIVISIONS

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INTRODUCTION

PART I of this study (GRELL 1946) contains an account of the somatic reduction divisions whereby during metamorphosis the highly polyploid multiple complex cells of the larval ileum and rectum of the mosquito are reduced to the tetra- or octoploid cells of the ileo-colon of the adult. Two types of division termed respectively first- and later-divisions are involved in this process of reduction. These division types, while similar in anaphase and telophase behavior, are remarkably different in prophase. The differences are probably correlated with the presence of a prolonged resting stage accompanied by repeated chromosome reduplication preceding first-division and a short resting stage or interphase with somatic synapsis instead of chromosome reduplication in later-divisions. It was proposed that by a comparison with ordinary diploid mitosis and with meiosis in Culex, the somatic reduction divisions might become more intelligible. In mitosis there is chromosome reduplication in the resting stage and somatic pairing of a primitive type. In meiosis, presumably, chromosome reduplication and synaptic pairing occur.

The present paper presents pertinent data regarding meiotic divisions as they occur in spermatogenesis, and diploid somatic divisions in the mid-gut, limb buds and nerve tissue of *Culex pipiens*. It likewise embodies conclusions and a discussion based on this and the first article of the series. The methods employed are described in Part I.

MEIOTIC DIVISIONS

Meiosis in *Culex pipiens* has previously been described by STEVENS (1910), LOMEN (1914), TAYLOR (1914), WHITING (1917), and MOFFETT (1936); hence only those points which have a direct bearing on the present problem will be discussed.

Spermatogonial prophase

In very early spermatogonial prophases the nuclear material appears as closely packed granular threads. A waviness suggestive of a relic coil (*cf.* arrow in fig. 1) may be seen. Doubleness of threads becomes apparent as the threads loosen (fig. 2, 3). This doubleness is the first indication of the somatic pairing of homologous chromosomes which characterizes the Diptera in general and which is present in various degrees in all divisions in Culex. Figure 4 represents the first stage in which the paired character of the homologous chromosomes can be traced throughout the length of the threads, and at which the haploid number, three, of paired elements can definitely be established. The pairing at this stage appears, not as an intimate side-by-side pairing as will be

seen later at pachytene (fig. 15), but as a series of relational coils. The pairing is complete except for particular areas, one in the mid-region of each pair of threads (fig. 4), where the chromosomes not only fail to pair but seem to repel each other. These unpaired regions are the flared regions previously described for the multiple complex cells (preceding paper). Figure 5 shows a later stage, with three pairs of polarized threads, each representing a pair of homologous chromosomes. As prophase progresses, the relational coils are gradually lost. The chromosomes assume the characteristic prometaphase and metaphase configurations, with homologous chromosomes no longer in actual contact, yet retaining their closely paired association (fig. 6). Chromosomes are seen to be longitudinally double at this stage which corresponds to the six-bundle stage of the first-division multiple complex cells in Culex.

EXPLANATION OF FIGURES

All photomicrographs are of *Culex pipiens*. Aceto-orcein smears. Magnification ×1250.

FIGURE 1.—Early spermatogonial prophase. Arrow points to relic coil.

FIGURE 2.—Early spermatogonial prophase.

FIGURE 3.—Early spermatogonial prophase. Note doubleness of threads.

FIGURE 4.—Spermatogonial prophase showing intimate pairing of homologous chromosomes into three groups. The arrows point to the flare regions where homologous chromosomes remain unpaired.

FIGURE 5.—Spermatogonial mid-prophase. The arrow indicates the position of the flare in one of the pairs of chromosomes.

FIGURE 6.—Spermatogonial prometaphase. Homologous chromosomes are paired but separate.

FIGURE 7.—Spermatogonial mid-anaphase. Note the pairing of homologous chromosomes.

FIGURE 8.—Spermatogonial anaphase showing paired homologous anaphase chromosomes.

FIGURE 9.—Spermatogonial late anaphase showing the six chromosomes grouped into three pairs.

FIGURE 10.—Very early first meiotic prophase.

FIGURE 11.—Synizesis-like stage of first meiotic prophase.

FIGURE 12.—Synizesis-like stage of first meiotic prophase.

FIGURE 13.—End of synizesis-like stage of first meiotic prophase.

FIGURE 14.—Transition from synizesis-like stage to pachytene.

FIGURE 15.—Pachytene. The arrow indicates the position of the flare in one of the bivalents.

FIGURE 16.—Early diplotene.

FIGURE 17.—Early diploid somatic prophase from larval brain. Three pairs of relationally coiled threads are seen.

FIGURE 18.—Mid-prophase of diploid cell from the imaginal ring of the larval hind-gut. Homologous chromosomes are closely paired.

FIGURE 19.—Mid-prophase of a diploid cell from the imaginal ring of a larval hind-gut. The arrow indicates the position of the SA-region.

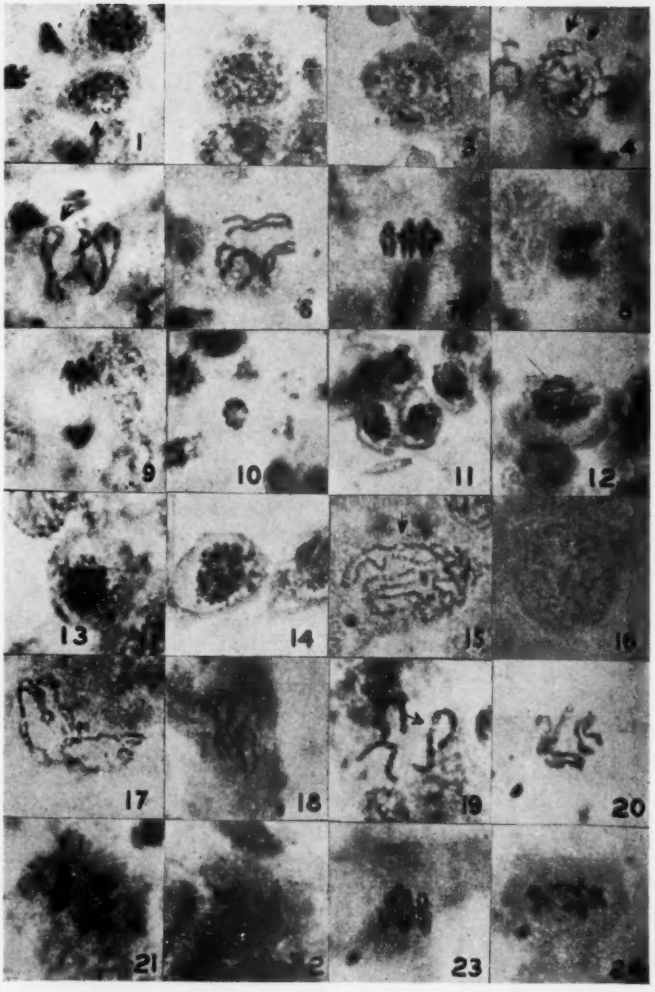
FIGURE 20.—Prometaphase of a diploid cell from the mid-gut of a pupa. Chromatids are visible.

FIGURE 21.—Metaphase of a diploid cell from a pupal mid-gut.

FIGURE 22.—Early anaphase of a diploid cell from the imaginal ring of a larval hind-gut.

FIG. 23.—Mid-anaphase of a diploid cell from a pupal mid-gut. Note the pairing of homologous chromosomes.

FIGURE 24.—Late anaphase, polar view, of a diploid cell from a pupal mid-gut. Note the paired arrangement of homologous chromosomes.



FIGURES 1–24

Spermatogonial anaphase

Somatic pairing is present throughout prophase and at metaphase when the chromosomes arrange themselves on the metaphase plate. Active somatic pairing, however, occurs as the metaphase chromatids separate and move to the poles as anaphase chromosomes. It consists in the coming together of homologous anaphase chromosomes en route to the poles. It is clearly seen in figures 7, 8 and 9, in which the three pairs of homologous chromosomes are clearly visible. Active somatic pairing at anaphase likewise occurs at every division of the multiple complex cells.

Meiotic prophase

As far as can be determined, the last spermatogonial telophase appears to give rise to a prolonged first meiotic prophase without an intervening typical resting stage. This early prophase takes the form of a synizesis-like figure, with a more or less knotted condition of the chromosomes (fig. 10, 11). A typical leptotene stage does not seem to occur. The threads appear to remain paired from the preceding anaphase or telophase. The gradual relaxation of the synizesis-like mass is accompanied by an increase in the size of the cell and the occasional appearance of paired threads (fig. 11, 12, 13). Actual synapsis probably occurs while the chromosomes are in the knotted mass.

There is to date no general agreement concerning early prophase of Culex. STEVENS (1910) began her account of the spermatocyte prophase with a description of the synizesis stage which in Culex, according to her, follows the parasynapsis of chromosomes which occurs in the preceding telophase. STEVENS was of the opinion that there was no necessary connection between synapsis and synizesis, but that probably synizesis was a period during which the chromatin material becomes modified in preparation for maturation. This conclusion was based on the observation that during the synizesis stage the staining qualities of the nucleolus changed in such a way as to indicate that it received chromatin material from the chromatic threads wound around it during this stage. WHITING (1917) maintained that the synizesis stage reported by STEVENS was an artifact. While WHITING does not specifically mention leptotene, he does report an early first spermatocyte nucleus with single and later with double threads. He believes that synapsis occurs at zygotene, without the intervention of a synizesis stage.

Reports regarding early first spermatocyte prophase behavior in the Diptera are to date extremely confusing and often contradictory. Much of this confusion is no doubt due to the presence of somatic pairing which makes the analysis of meiotic pairing more difficult. SMITH (1942) elaborated a generalized theory of pairing at premeiotic telophase in the Diptera. This pairing he held to be conditioned by the singleness of chromosomes at this stage. This may possibly be the case in Culex. As far as can be determined, the anaphase and telophase chromosomes are optically single and pair in the last premeiotic division as well as in any other spermatogonial division. COOPER (1944) found it impossible to resolve the initial stages of meiotic prophase in Olfersia. He is of

the opinion, however, that the lack of early synaptic stages does not seriously affect the interpretation of the meiotic mechanism, since "it is becoming increasingly clear that true leptotene stages may be absent in flies. . . ."

It appears certain that meiotic prophase in the Diptera differs in several respects from the classical meiotic procedure. The presence or absence of a synizesis stage following somatic pairing and its correlation with synapsis seem to be such points of difference. In the present investigation the term "synizesis-like" is used for the knotting of chromosomes, to avoid confusing this stage with the synizesis stage commonly reported in other organisms.

As the synizesis-like knot of Culex definitely loosens, the paired, pachytene chromosomes become evident (fig. 14, 15). In cells at this stage synapsis, presumably a gene-for-gene association, appears to be complete except at restricted regions which seem to correspond to the flared regions described earlier for spermatogonial prophases (compare fig. 5 and 15). Pachytene chromosomes are optically single in Culex, chromatids becoming visible during diplotene (fig. 16).

DIPLOID SOMATIC DIVISIONS

Diploid somatic divisions are found in relative abundance in the mid-gut, brain, ganglia, limb buds, and anterior imaginal ring of the ileum of the mosquito. Although these diploid mitoses have previously been described, the points which are of particular significance in the present study—namely, early prophase behavior and anaphase pairing—have in each case received rather inadequate treatment. They are included here for the purpose of comparison with other division types in Culex.

Diploid somatic divisions are essentially similar to spermatogonial divisions. From early prophase (fig. 17) and through mid-prophase (fig. 18, 19) the chromosomes appear as three pairs of relationally twisted threads. With the development of a somatic coil and a corresponding contraction, the threads lose their relational coil, so that by prometaphase the members of a pair lie free (fig. 20). Chromatids are visible at this stage. At metaphase (fig. 21) the chromosomes though not in actual contact nevertheless retain the paired relationship described by METZ (1916) for the Diptera in general.

Anaphase separation affects first the SA-regions and then the paranemically coiled chromatid arms (fig. 22). En route to the poles homologous chromosomes again pair (fig. 23). The pairing continues into telophase, through the resting stage, and reappears in the polarized chromosomes of the following prophase. Reduplication of threads presumably takes place during the resting stage. Evidence for this is obtained by a comparison with first-division multiple complex cells of the ileum, in which reduplication is known to occur in the resting stage.

Pairing in diploid somatic cells

At no time in the mitotic cycle of Culex are homologous chromosomes unpaired. At metaphase and early anaphase the pairing is least intimate. Active

pairing of anaphase chromosomes takes place as the chromosomes move to the poles side by side. Figure 24 is a polar view of late anaphase chromosomes showing the somatic pairing of homologous chromosomes. This pairing apparently becomes more intimate at telophase as the threads become partially despiralized. At some time between the disappearance of distinct threads in telophase and their reappearance in prophase, the parallel, paired relationship becomes one of relationally twisted threads of prophase. A comparison with multiple complex cells (Part I of this study) indicates that the formation of relational coils is probably associated with chromosome reduplication, rather than with pairing mechanisms. In general appearance homologous chromosomes seem to be most intimately paired in mid-prophase (fig. 19) when the relational coils are still present and the new somatic coil is forming. Together they give the paired chromosomes the general appearance of a single heavier chromosome.

As in multiple complex first-division prophase, spermatogonial mid-prophase, and pachytene of meiosis, the pairing in these somatic prophases is complete except for the flared regions where threads appear to repel each other. These are present in the mid-regions of each of the three pairs of homologues (fig. 18, 19).

Coiling in diploid somatic cells

Relic, somatic, and relational coils are found in the prophase of somatic mitotic divisions. As mentioned above, the relational coils are present from the beginning of prophase to prometaphase (fig. 17–20). The somatic coil is probably effective in contracting the paired chromosomes, first to the much shortened mid-prophase condition (fig. 19) and then to the maximum contraction of metaphase (fig. 21). The somatic coils of the diploid somatic chromosomes do not compare in clarity with those of the first-division multiple complex prometaphase and metaphase chromosomes in Culex. The presence of of a relatively marked relic coil in diploid cells and its absence in first-division multiple complex cells is significant. In the multiple complex cells the long resting stage during which multiple chromosome reduplication occurs allows time for the relic coil to disappear almost completely. In diploid somatic cells there is presumably a single reduplication of threads, with a shorter resting stage relative to that in the multiple complex cells. It is probable that with each additional chromosome reduplication the threads progressively lose more of the relic coil.

SA-regions in diploid somatic cells

Reference to figure 19 reveals a non-staining segment adjacent to the flared region which is in all probability the SA-region. This conclusion is warranted on the basis of a comparison with corresponding prophase stages of first-division multiple complex cells where the SA-regions appear as achromatic areas at one end of the flared regions. It is possible that in the diploid somatic, as in the multiple complex chromosomes, the SA-regions of the homologous chromosomes have fused.

DISCUSSION

Somatic reduction

Somatic reduction is intimately linked with somatic pairing. That this pairing alone does not cause reduction in the multiple complex cells is clear from a comparison of these cells with diploid somatic and spermatogonial divisions. In multiple complex cells the active pairing at anaphase is followed by a reduction in chromosome number. In diploid somatic cells somatic pairing occurs at anaphase, yet reduction does not follow. Somatic pairing at anaphase of the final spermatogonial divisions, while apparently similar to that in diploid somatic cells, is followed by a reduction in chromosome number in the ensuing meiotic divisions. Whatever is responsible for these differences in subsequent behavior in the three division types mentioned must be effective after anaphase somatic pairing which is common to all. The present study has confirmed the opinion of previous investigators that somatic pairing occurs in every diploid division in Culex and has extended this idea to all the divisions of the multiple complex cells as well.

Both of the types of multiple complex divisions, first- and later-divisions, described in Part I of this study, may be called reduction divisions. The actual numerical reduction, however, becomes effective after the first-division. Separation of metaphase chromatids at anaphase of first-division is similar to chromatid separation in any ordinary mitotic division in Culex. Multiple complex cells, however, undergo reduction while ordinary cells do not. The essential difference is that in the resting stages or interphases following the first-division of multiple complex cells there is no reduplication of threads such as occurs in the resting stage between all other somatic mitoses and restores the number of chromatids which was halved at the previous anaphase. Every mitotic division would, therefore, be a reduction division if there were not a succeeding reduplication. In like manner, multiple complex first-divisions would resemble ordinary mitotic divisions if there were a reduplication of threads in interphase. In meiotic divisions where chromosome reduction is the common occurrence there is chromosome reduplication preceding or accompanying first division, but as in the multiple complex cells, there is no reduplication in interphase. In the multiple complex cells then as in meiosis, actual numerical reduction occurs in the second division.

BERGER (1938), aware of the differences between ordinary somatic and multiple complex divisions, suggested a mechanism for somatic chromosome reduction. This mechanism has received supporting evidence in the present study. HOLT (1917) described a parasynapsis between the first and subsequent divisions in the multiple complex cells and suggested that probably the synapsed threads separate again at the next prophase. Her descriptions leave no doubt that she referred to typical somatic pairing and not to a phenomenon that would result in reduction. In the present study cytological evidence for somatic reduction is based on the striking differences which exist between first- and later-divisions of the multiple complex cells, and between these and other division types.

In multiple complex first-divisions the chromosomes are conspicuously and loosely bound together into three bundles from earliest prophase. A prophase bundle of this kind consists of relationally twisted homologous elements, each composed of a group of sister chromosomes themselves relationally coiled in two's. In diploid cells homologues are likewise relationally bound together into three groups, but here each homologue consists of a single chromosome. Relational coiling of homologues into the haploid number of bundles does not occur in later-divisions. Instead, the chromosomes are arranged in three more or less distinct groups in each of which the polarized chromosomes (homologous or sister) loosely approximate each other without coiling. The absence of relational coiling between the homologous or sister chromosomes in later-division groups may be due to the failure of threads to reduplicate preceding later-divisions. The presence of polarization in later-division and its absence in first-division may be explained by the long resting stage with chromosome reduplication that precedes the first- but not the later-divisions. Correlated with the fact that later-division is unique in its absence of relational coiling is the interesting observation that later-division is likewise unique in the absence of reduplication preceding prophase. This difference in the behavior of the later-divisions supports the idea of a pairing and subsequent separation of threads previously formed, rather than a separation of threads reproduced during interphase, as is the case in the other cell types.

Another striking difference between multiple complex and other division types is the obvious dissimilarity in length of the threads at corresponding stages of division. In the multiple complex cells, at no time during prophase do the later-division chromosomes approach in length those of first-division. While it is not practicable to make exact measurements of early prophase chromosomes, it is estimated that early diploid somatic prophases are longer than later-division ones and that meiotic prophase chromosomes (pachytene bivalents, the earliest stage at which approximate length can be determined) fall between first-divisions and diploid somatic divisions. Later-division prophase chromosomes are thus seen to be relatively the shortest of the four types. This seems logical if it is granted that with repeated reduplication of threads there is a progressive uncoiling of the chromosomes, the degree of despiralization being in part correlated with the number of reduplications which occur. Multiple complex first-division chromosomes which have undergone several chromosome reduplications to produce the 4n to 64n condition would at that rate have attained a considerable increase in length over later-division chromosomes which do not reduplicate. Preliminary observations on the salivary gland chromosomes of Culex are in general agreement with this interpretation. These giant chromosomes by far exceed in length the first-division multiple complex chromosomes. Concomitantly these giant chromosomes appear to undergo more equivalent reproduction of genetic material. Chromosomes in diploid cells which presumably undergo one chromosome reduplication preceding each division are logically much shorter than corresponding multiple complex first-division chromosomes and longer than later-division chromosomes. This is in agreement with actual observations.

Chromosome reduplication does not account for the length of meiotic prophase threads relative to that of diploid cells. The supplementary factor in meiosis may be complete or almost complete despiralization in preparation for the gene-by-gene approximation of synapsis. The cause of this uncoiling is not immediately clear. As mentioned above, first-division multiple complex prophase chromosomes appear to be longer than meiotic prophase chromosomes. If this observation is correct, and if meiotic prophase threads have undergone complete or almost complete despiralization, a factor other than despiralization must account for the greater length of these multiple complex chromosomes. BUCK (1937) and others account for the unusual increase in the length of the salivary gland chromosomes on the basis of the addition of material, probably between the chromomeres. There is at present no evidence for this method of growth in the multiple complex cells.

While later-division prophase chromosomes are shorter than those in other division types, a certain amount of increase in length does occur. In the absence of reduplication, another factor correlated with increase in length must be postulated. This factor may be somatic synapsis.

Somatic synapsis is a term used by PAINTER (1934) to describe the intimate union that homologous chromosomes or parts undergo in the nuclei of the salivary glands of Diptera. The pairing is line for line and band for band, and the two homologous chromosomes unite into one apparently single structure. PAINTER, DOBZHANSKY and TAN (1936), and BUCK (1937) have distinguished between somatic pairing and somatic synapsis. DOBZHANSKY and TAN distinguished between the intimate pairing found in pure species of *Drosophila pseudoobscura* and *D. miranda* and the less intimate pairing of the hybrid form of these species. PAINTER suggests that both somatic pairing and somatic synapsis may be the expression of the same force. The already confused state of terminology regarding pairing in the Diptera makes one hesitate to introduce another term, yet this would seem to be necessary to express unequivocally the condition that exists between threads in later-division prophases of multiple complex cells. In the present study no new term has been introduced. PAINTER'S suggestion that somatic pairing and somatic synapsis may represent different degrees of the same phenomenon seems to justify the use of the term somatic synapsis for the association of threads in these later-divisions. The term somatic synapsis, therefore, has been used for the phenomenon whereby threads that have undergone somatic pairing at anaphase enter upon a more intimate union in the process of reduction.

Somatic synapsis in later-divisions while intimate in the sense of involving a very close approximation of sister or homologous threads is presumably not a gene-by-gene association judging from the length of the threads and the obvious doubleness seen in well smeared preparations. The association is closer than somatic pairing but less intimate than meiotic synapsis. Presumably the synapsis which occurs is effective while the threads still retain to some extent their previous somatic coil. Later-division prophases as a rule have a well defined relic coil.

Somatic synapsis of threads which underwent somatic pairing in the previous anaphase, despiralized somewhat in the ensuing telophase, and passed through a very brief interphase, seems to occur at or near the onset of later-division prophase. The synapsed chromosomes appear superficially as one unit until mid-prophase when the constituent halves, now called later-division chromatids, open out in preparation for separation at anaphase. Anaphase separation in later-divisions, therefore, separates somatically synapsed homologous chromosomes. This constitutes the mechanism of somatic reduction which was suggested by BERGER (1938) and which is substantiated here by means of a comparison of the two types of multiple complex divisions with meiotic and ordinary diploid mitotic divisions. In later-divisions, then, there is somatic pairing, followed by somatic synapsis without an intervening reduplication, leading finally to somatic reduction in chromosome number.

Pairing

Attempts to find a single factor responsible for pairing in meiotic and mitotic cycles have to date led to no useful conclusion. Since the formulation of the precocity theory by DARLINGTON (1931), alternative explanations have been proposed to account for pairing not in accordance with conditions stipulated by him, but in no case have all the known facts concerning pairing been fully explained. DARLINGTON's hypothesis of attraction in two's only, while stimulating, is by no means generally accepted. The discovery of somatic pairing (STEVENS 1908, 1910; METZ 1916) and of somatic synapsis (PAINTER 1933, 1934) in the Diptera have increased the difficulty of reducing pairing phenomena to a common cause.

PAINTER (1933) believes that the forces which cause homologues to unite in the salivary gland are probably the same as those which cause homologues to unite in meiosis. SCHRADER (1944) leans to the view that meiotic pairing involves no special forces other than those active in ordinary mitosis, the fundamental difference being one of timing and emphasis. Others prefer to postulate more than one force to account for meiotic and mitotic pairing. Thus, KING and BEAMS (1934), working on the salivary gland chromosomes of Chironomus, suggest that probably two forces are active. One of these causes homologues to pair. It is active at meiosis in most organisms and at all times in Diptera. The other force causes homologues to disjoin. It is active both in meiosis and in the Diptera where it forces homologues apart at metaphase.

Somatic pairing has been variously explained. WHITE (1937) attempts to explain it on the basis of a residual attraction which consists in forces remaining over after prophase and metaphase pairing have been satisfied. This suggests the specially exaggerated property of attraction which DARLINGTON (1937) proposes as being effective at mitosis in diploids of many Diptera. METZ (1906) attributes somatic pairing in diploids and tetraploids to a likeness in constitution of corresponding chromosomes. COOPER (1938) maintains that somatic pairing results from the mutual attraction of homologous loci, the degree of pairing depending on the degree of approximation of homologous regions. On

that assumption pairing would be correlated with the amount of uncoiling of the chromosomes. A high degree of uncoiling would then appear to be the essential prerequisite to synapsis for both meiotic and somatic chromosomes in the Diptera. COOPER attributes synapsis in polytene chromosomes to two factors, uncoiling of chromosomes and forces of somatic pairing. The opinion that somatic pairing and somatic synapsis (PAINTER 1934; PAINTER and GRIFFIN 1937) may in part be considered different degrees of expression of the same phenomenon has served as a convenient working hypothesis for the present interpretation of pairing.

Culex, manifesting as it does a variety of relationships, is a fertile source of information regarding pairing. Any single theory destined to account for all the pairing observed in Culex must explain the following diverse expressions of pairing seen in meiosis, diploid mitosis, and multiple complex cells: (1) The relationally coiled condition of homologous chromosomes in early and midprophase of ordinary mitotic and gonial divisions, resulting in the haploid number of pairs. Each homologue here consists of two chromatids. (2) The relationally coiled condition of homologous chromosomes in early and midprophase of first-division multiple complex cells, resulting in the haploid number of prophase bundles. Each homologue here consists of a multiple number of sister threads, themselves paired and relationally coiled in two's, and in pairs of two's. (3) The side-by-side, but not touching, pairing of longitudinally double homologous chromosomes at prometaphase and metaphase in diploid and gonial divisions, resulting from the loss of the relational coils uniting prophase pairs. In first-division multiple complex cells there is a corresponding association of longitudinally double chromosomes in six groups of from two to 24 sister chromosomes each. In these sister chromosomes the chromatids may be completely separate even at the SA-regions, yet remain laterally associated without chiasmata or other visible connection. (4) Active somatic pairing of optically single homologous chromosomes at anaphase of every division, whether premeiotic, ordinary mitotic, or multiple complex division. (5) Active somatic pairing followed by somatic synapsis of optically single homologous or sister threads in preparation for later-division separation of chromosomes in multiple complexes, and the simultaneous absence of evident relational coiling. (6) The appearance of a haploid number of later-division chromosome groups, each consisting of loosely arranged but not relationally coiled later-division chromosomes. (7) Meiotic synaptic association in which in Culex the pairing as seen in pachytene is complete along the length of the chromosomes, except for the flared regions. Apparently no leptotene stage occurs; the paired condition seems to continue from premeiotic anaphase to meiotic prophase. (8) Diplotene and diakinesis bivalents with pairing maintained in the presence of chiasmata. (9) Flared regions of ordinary mitotic, meiotic, and multiple complex prophase chromosomes, representing localized regions where the homologous and sister chromosomes seem to repel each other. While at first glance these pairing conditions appear very different, closer analysis reveals several significant basic modes of behavior.

At every anaphase in Culex, with the exception of first and second meiotic anaphase, homologous or sister chromosomes undergo active pairing. Doubleness of anaphase chromosomes has not been seen in this material, nor has it to the author's knowledge previously been reported for Culex. On the assumption then that anaphase chromosomes are in this case single, there is at every anaphase an active pairing in two's of single chromosomes. This common factor in all divisions, whether mitotic, premeiotic or multiple complex, serves as a convenient starting point for a comparative analysis of conditions of pairing. The following résumé, while based on actual observations in Culex, includes the author's interpretation of how the pairing phenomena found in mitotic, somatic reduction, and meiotic reduction divisions may be correlated.

Active pairing at anaphase has been conclusively established for Culex. This pairing continues into telophase. The paired telophase condition merges imperceptibly into the resting stage in cases where a resting stage occurs. The crucial differences between somatic reduction, meiotic reduction, and mitotic phenomena most probably reside in this resting stage or in the very early succeeding prophase. This assumption is based on evident differences of prophase behavior in the various types of division.

During the resting stage following active anaphase pairing of homologous chromosomes in any type of division in Culex, one of two possibilities must occur: either (1) reduplication of threads does not take place; or (2) reduplication of threads takes place. Evidence as to which of these alternatives exists in a particular instance is found in the behavior of threads in the succeeding prophase. The following interpretation is here proposed as a basis for distinguishing between the types of prophase behavior. When reduplication of threads does not occur during the resting stage, the active pairing begun at anaphase continues uninterruptedly into the following prophase and results in somatic synapsis in the case of later-division multiple complex cells, or, on the assumption that reduplication of threads does not occur until pacyhtene, in meiotic synapsis in the case of meiotic cells. Somatic synapsis occurs at the end of interphase or in very early later-division prophase of the multiple complex cells. To all appearances the threads which synapse following the initial anaphase somatic pairing are optically single. The unique behavior of these synapsed threads supports the conclusion that no reduplication has in this case occurred. The despiralization of later-division chromosomes is not complete. By comparison with meiotic synapsis there does not appear to be a gene-by-gene association, but rather one that will allow of more ready separation at the succeeding anaphase. It is of interest to note in this respect that relative lengths of prophase stages in meiotic and later-division multiple complex divisions lend support to the view that there is gene-for-gene association in the one case and not in the other. BEASLEY (1938) lists the comparatively long duration of meiosis as one of three factors which work harmoniously to allow for intimate gene-by-gene association. This same idea formed the essence of the retardation theory of SAX and SAX (1935). This theory is based on differences in the chromosome lengths in mitotic and meiotic prophases, and differ-

ences in duration of prophase in mitotic and meiotic cycles. Meiotic prophase, being longer, allows for more complete despiralization. In Culex meiotic prophase is long, and later-division prophase is short. An intimate pairing in the latter is thus prevented.

Meiotic synapsis in Culex is difficult to follow. After premeiotic anaphase pairing, the chromosomes aggregate in a more or less dense mass which later opens out as a pachytene nucleus. It is questionable whether this intervening stage ought properly be called a resting stage. Apparently chromosome reduplication occurs during this time, but whether it precedes or follows actual synapsis is a matter of conjecture. Reduplication probably occurs after synapsis. Besides the occurrence of reduplication, meiotic cells undergo chiasma formation. In both these respects meiotic material differs from cells undergoing somatic synapsis. It seems justifiable, however, to consider meiotic synapsis and somatic synapsis as expressions of the same force, existing in different degrees or acting over different periods.

The second possibility to account for the different types of prophase behavior is based on the fact that in some cases reduplication of threads occurs in the resting stage following anaphase pairing of optically single threads. If reduplication occurs, active pairing ceases and despiralization of the relational coils begins. Differences in subsequent prophases have revealed that either the chromosomes undergo one reduplication, as in diploid somatic and spermatogonial mitoses, or they undergo multiple reduplication to form the multiple complex cells. In both instances reduplication is reflected in subsequently appearing relational coils between homologous chromosomes. In meiosis relational coiling does not appear until after the threads have doubled. In later-divisions of the multiple complex cells relational coiling is absent. Concomitantly, these cells do not undergo chromosome reduplication. The resting stage preceding diploid somatic mitosis is short relative to the multiple complex first-divisions. Reduplication presumably occurs before the chromosomes have undergone complete despiralization of the previous somatic coil, thus preventing a gene-by-gene association of homologues. The new relational coil probably arises with reduplication. Repeated chromosome reduplication must occur during the long resting stage preceding first-division of multiple complex cells. The long period during which these reduplications occur allow for complete or almost complete uncoiling of the somatic coil, with a resulting chromomere-by-chromomere association. In the first-division prophase bundles the fused regions give evidence of this close association.

In diploid somatic and spermatogonial mitoses and in multiple complex first-divisions active pairing which in meiotic and later-divisions continued on to synapsis is stopped by chromosome reduplication. This seems to occasion relational coiling. All subsequent relationships between the homologous units, whether multiple or not, seem to be an unpairing process, resulting finally in the complete loss of relational coils before late prophase. Unpairing occurs not only between homologous units, but in the first-division multiple complex prophases between sister chromosomes also. It is during this unpairing of sister

chromosomes that sister pairing in two's becomes especially clear. As the sister chromosomes fall apart, it becomes evident that the pairing is in two's, in pairs of two's, and in pairs of such pairs. The most recently reproduced threads are the chromatids. They remain in close association throughout prometaphase and metaphase, even when the SA-region has divided previous to prometaphase. This lateral association with no visible connection between the threads represents the highest degree of somatic pairing. It is present in a smaller degree in those prometaphase chromatids where the arms but not the SA-regions have divided before late prophase bundles fall apart. This type very closely resembles diploid somatic and spermatogonial prometaphase chromosomes. Essentially the mode of behavior appears to be similar in diploid somatic, spermatogonial, and multiple complex first-division cells.

A superficial comparison of diploid somatic and spermatogonial prophases in which homologue pairing is effective between longitudinally double threads, and multiple complex first-division prophases where pairing is effective between homologues consisting of a multiple number of threads leads to the conclusion that both these cases offer arguments against the pairing in two's proposed by DARLINGTON as one of the essentials of his precocity theory. Closer analysis proves that this is not the case. Actually pairing is effective in two's. To ascertain this, it has been necessary to go beyond the gross association and to consider the smallest subdivision of the chromosome groups in order to determine the fundamental pairing relationship. It will be recalled from the description of the multiple complex and diploid somatic cells that homologue somatic pairing results from an active anaphase pairing of two optically single threads. As these homologues reduplicate and a relational coil forms, the unpairing begins. Chromatids, then, are paired in two's, while the homologues as such are already in the process of unpairing. Sister somatic pairing results from the reduplication of threads in the resting stage. As each thread becomes double, the first formed pairs are more widely displaced relative to each other, the loose association between them being retained as long as the relational coils are present. Actual pairing is always maintained between the most recently reproduced threads, the chromatids.

Flared regions of the chromosomes

The localized modifications of the chromosomes referred to in this paper as flared regions have not previously been reported for Culex. They are consistently present in the mid-region of each pair of homologous or sister chromosomes or groups of chromosomes in spermatogonial, diploid somatic, meiotic, and multiple complex first-division prophases. Later-division multiple complex and second meiotic prophases are unique in the absence of noticeable flares. In gonial, diploid, and meiotic cells the flared regions represent the spreading apart of two paired chromosomes. In multiple complex prophase the flare is effective, first between entire homologous groups, and superimposed on this, between the sister chromosomes comprising each group. In all except the multiple complex cells the flares disappear as homologue pairing is undone. In

the multiple complexes the flares between sister chromosomes are retained until prometaphase when sister-groups fall apart.

METZ (1922) described a situation in the robber fly, *Dasyllis grossa*, which may be analogous to the flares in Culex. In three of the five pairs of chromosomes of Dasyllis there are unpaired, usually median loops present in both spermatocytes and in oocytes, and to a less marked degree in spermatogonia. METZ suggested incomplete synapsis as the factor determining the unpaired loops. The observation that three of the chromosomes were similarly affected led METZ to conclude that the unpaired loops were affected by the general synaptic process rather than by the independent action of agents located in the respective pairs. In Culex all the chromosomes of a set show a flared region. It is possible that here, as in the robber fly, some general synaptic or other pairing phenomenon is responsible for the flare between homologous units. However, the appearance of flares between sister chromosomes in multiple complex cells which do not undergo active pairing are not explained unless it is assumed that repulsion forces residing in the unpaired regions would tend to separate sister chromosomes as they are reproduced. Flares have not been observed between chromatids.

COOPER (1944) described a central or kinetochore-containing loop, and adjacent to this a second loop in the long arms, in the V-shaped, somatically paired autosomes in Olfersia. In Culex the flared region does not include the kinetochore region but is in each case immediately adjacent to it. It seems quite unlikely, judging from the descriptions and figures given for Olfersia, that the localized non-pairing regions in it and in Culex are similar in nature. Likewise, there is question whether the condition described by COOPER (1941) for *Melophagus ovinus* in which in the small autosomes both ends appear to be paired while the mid-regions appear to repel each other, is equivalent to the flared regions in Culex.

In the salivary gland nuclei of Chironomus, POULSON and METZ (1938) report swollen regions of chromosome IV, associated with the nucleolus. These swollen regions represent parts of the chromosomes in which the banded structures of the chromosomes are distinctly modified. The same authors (1938) describe a variable number of "puff" regions distributed through the salivary gland chromosomes of *Sciara ocellaris* in which normal banding is likewise disrupted. The "puffs" may or may not be present in a particular individual. If they are present, they appear simultaneously in all the nuclei. POULSON and METZ could find no true nucleolus in Sciara. They suggest, however, on the basis of the modified banding character of the "puffs" that the swollen regions may be modified directly to form the nucleolus-like regions, while the nucleoli appear in intimate relation with similarly banded regions in Chironomus. These differences between Chironomus and Sciara indicate a variation in the distribution of chromatic and achromatic material in the two forms. In Culex the regular occurrence of flares in each of the three pairs of chromosomes of a set makes the association of these regions with nucleolar material seem doubtful. The observation of satellites on one of the chromosome pairs indicates

that this pair is alone concerned with the formation of the nucleoli and that the flared regions are localized unpaired regions.

SA-regions

In spite of the large size of the fused SA-regions of Culex during part of the multiple complex division cycle, structures similar to the commissural cup and spindle spherules in certain Amphibian kinetochores (SCHRADER 1936, 1939), the Leitkörperchen in nuclei of various Amphibia (METZNER 1894), and angiosperms (TRANKOWSKY 1930), or the kinetonema of *Trillium kamtschiaticum* (MATSUURA 1941) have not been seen. Since differential fixatives were not employed, the failure to observe structures in the primary constriction region in no way eliminates the possibilities that spherules or kinetonema may be present. The compound character of the SA-regions of homologous groups of chromosomes in the multiple complex first-division prophases makes it seem probable that these regions may be favorable for a more detailed study of kinetochore structure. In aceto-orcein smear preparations the SA-regions in the chromosomes of Culex appear consistently as clear, achromatic regions. They correspond in their apparent lack of structure to similar achromatic kinetochores reported for pachytene chromosomes of Zea (MCCLINTOCK 1933) following acetocarmine staining.

The SA-regions of early and mid-prophase of first-division multiple complex bundles appear to be single in Culex. It will be recalled that each such prophase bundle consists of two homologous groups of chromosomes closely paired since the previous anaphase. Whether the spindle attachment regions of homologous chromosomes actually fuse, or whether the apparently undivided condition of the prophase SA-regions is the result of a matrix common to both homologous chromosomes it is impossible to decide. The apparent fusion may be analogous to the fusion which MCCLINTOCK (1933) observed between the spindle attachment regions of two pachytene bivalents in Zea and which she attributed to a stickiness which these regions possess at this stage of meiosis. A similar property in the SA-regions of Culex is not entirely ruled out.

Whereas SA-regions between homologous units are single as a result of an apparent or real fusion, those between sister chromosomes of first-division multiple complex cells are single during early and mid-prophase presumably because they have not as yet divided following chromosome reduplication in the resting stage. The time for the division of these SA-regions is not fixed. The variations in the condition of the chromosomes at prometaphase of the multiple complex first-divisions, ranging from chromatids held together at the SA-regions to chromatids completely separated, indicate that the time of division of the spindle attachment regions is not restricted to a particular stage and that it may occur before metaphase. In Culex the SA-regions between chromatids may thus divide before, during, or after prometaphase. In cases where the division occurs before or during prometaphase, sister chromatid SA-regions may begin to separate before actual metaphase orientation. This is in harmony with the idea that there is mutual repulsion between SA-

regions during certain stages of division, and that forces initiating separation are localized in the chromosome itself. This conclusion agrees with that of HUGHES-SCHRADER (1943) who describes similar autonomous kinetochore movements in meiosis of several species of mantids.

Endomitosis

In slides of the mosquito ileum sent to PAINTER by BERGER, PAINTER and REINDORP (1939) reported finding evidence for an endomitotic process similar to that described by them (1939) for Drosophila and by GEITLER (1939) for Gerris. In the present study no such evidence was found. Rather, results are in agreement with the statement of BERGER (1937, 1938) that throughout their growth period the cells of the ileum maintain the typical resting stage character. In Drosophila nurse cells PAINTER and REINDORP give convincing evidence for a process similar in essentials to that described by GEITLER (1939)—that is, endomitosis in the strict sense of the term involving endoprophase, endometaphase, and the succeeding stages. But PAINTER and REINDORP's additional comment that from a "broader point of view" findings regarding the multiple strand concept of salivary gland chromosome structure and the mosquito multiple complex cells are in entire accord with the observations on the nurse cells of Drosophila are less convincing. Furthermore, the multiple complex cells are not in accord with a later description of endomitosis (BIESELE, POYNER, and PAINTER 1942). During endomitosis, according to this later description, "There is a coiling of the threads, a pronounced increase in chromaticity, a slight separation of chromatids, and subsequently an uncoiling of the threads to form the usual resting nucleus, which assumes a volume twice that which it previously had." That an internal reduplication of chromosomes does occur in Culex may now hardly be doubted, but that the process even from a "broader point of view" ought to be considered as endomitosis in the strict sense is questionable.

In a recent paper WITKUS (1945) draws a clear distinction between the various methods whereby polyploidy may be produced by chromosomal reduplication without nuclear division in both plant and animal material. "Endomitosis," she says, "is only one of three known methods by which this can occur, although the term has been used to describe all three procedures in some of the recent cytological literature." As perhaps the clearest example of the first type she lists the production of highly polyploid cells in the ileum of Culex by repeated reduplication of chromosomes in the resting nucleus. The second method consists of double reduplication in the resting stage, as seen in Spinacia (GENTCHEFF and GUSTAFSSON 1939a, 1939b; BERGER 1941). Only in the third type, endomitosis proper, are there endoprophase, endometaphase, endoanaphase, and endotelophase stages. WITKUS suggests that probably the salivary gland chromosomes of the Diptera should be placed under type one.

Endomitosis considered in the sense in which it was originally defined by GEITLER is thus distinct from types in which, as in Culex, chromosome reduplication occurs while the nucleus retains an apparently resting stage char-

acter. Present evidence points to the conclusion that in the ileum of *Culex pipiens* endomitosis does not occur.

SUMMARY

Flared regions similar to those found in multiple complex first-division chromosomes are found in diploid somatic, in gonial, and in meiotic prophases. They are absent in later-division multiple complex prophases and in second meiotic divisions, and they do not occur between chromatids.

Somatic pairing of homologues is found throughout the mitotic cycle in spermatogonial and diploid somatic as well as in multiple complex cells. It is kept in effect by an active coming together of homologues at every anaphase.

A comparison of prophase chromosome behavior in the various types of division indicates that (1) if chromosome reduplication occurs in the resting stage, active pairing stops and a process of unpairing begins, (2) if chromosome reduplication does not occur or is delayed, the active somatic pairing begun at anaphase continues into somatic synapsis in the one case and meiotic synapsis in the other.

In Culex both active pairing and that resulting from multiple chromosome reduplication seems to be closest between two optically single threads. When more than two threads are present, there is a gradual relaxation and unpairing of pairs but no evidence of repulsion.

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THE SELECTIVE ELIMINATION OF INVERSION DICENTRIC CHROMATIDS DURING MEIOSIS IN THE EGGS OF SCIARA IMPATIENS

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INTRODUCTION

THE recent studies of CARSON (1944) and MCCARTHY (1945) have shown that, in certain species of the dipteran genus Sciara, chromosome inversions are commonly encountered in natural populations. This fact has been obscured until recently because the species which have been most extensively studied, such as *Sciara ocellaris*, *S. reynoldsi*, and hybrids between them, show salivary gland chromosome changes almost exclusively of the duplication-deficiency type. In *Sciara impatiens*, on the other hand, the writer (1944) has described numerous long inversions in each of the four chromosome pairs. The gene sequences resulting from these inversions may be arranged in a phylogenetic series for each chromosome. These phylogenies, moreover, are unidirectional—that is, one sequence can be designated as ancestral and the remaining ones can be shown to be derived from it and from each other in a stepwise fashion.

Long inversions should theoretically be subject to adverse selection, since it is well known that most types of crossing over within the confines of an inverted segment in inversion heterozygotes will lead to disturbances at meiosis which result, under certain conditions, in partial sterility. The derived arrangements in *Sciara impatiens*, however, appear to be at no selective disadvantage as compared with the ancestral ones. Natural inversion heterozygosity is very high, and almost all the rearrangements are universally encountered in natural populations from widely distributed areas in the eastern United States, even when population sampling is small. The presence of some mechanism which counteracts the expected sterility is thus inferred.

In *Drosophila melanogaster*, very little, if any, inviability accompanies paracentric inversion heterozygosity, and this has been considered to be due not to any suppressive activity of the inversion on crossing over, but rather to the mechanical elimination of dicentric and acentric chromatids from the definitive female pronucleus during meiosis. Thus STURTEVANT and BEADLE (1936) have presented the following hypothesis which is based on their own genetic studies of paracentric inversions in the X chromosome and the geometrical relations of the meiotic divisions in the egg as described by HUETTNER (1924). Crossover chromatids with two centromeres are said to form a dicentric bridge or tie between the two nuclei at the first meiotic division. This bridge presumably remains in this position as the nuclei are reorganized for the second divisions. Since the axes of the second division spindles lie in a straight line, it is assumed that the non-crossover chromatids disjoin from the centromeres of the dicentric and move into the terminal nuclei, one of which is the oötid

nucleus. In this manner, recovery of most types of crossovers would be suppressed and possible egg inviability effectively prevented. STURTEVANT and BEADLE have suggested that this situation may prevail widely in oögenesis in animals and in megasporogenesis in higher plants. That first division bridges do operate in this manner in megasporogenesis of certain hybrid plants (Lilium, Tulipa) has been demonstrated by DARLINGTON and LA COUR (1941).

The purpose of the present paper has been to verify this scheme cytologically for the dipteran egg in the meiotic divisions of the eggs of *Sciara impatiens*. This material is especially favorable for a number of reasons. In the balanced stock used (see Material and Methods), half of the females carry an extremely long inversion in the heterozygous condition, whereas the other half, which serve as controls, carry no chromosome rearrangements and are homozygous for the standard arrangements of all chromosomes. Large numbers of accurately timed eggs can be obtained with ease, and the chromosomes are sufficiently large and few in number to permit a detailed cytological analysis. The fact that the chorion of the Sciara egg is delicate and transparent makes its removal unnecessary, and the eggs are small enough so that they can be stained by the Feulgen technique and studied as whole mounts.

A selective elimination of dicentrics and fragments, concomitant with a directed segregation of a non-crossover chromatid into the oötid nucleus regularly occurs; the process is in close agreement with the hypothesis of STURTEVANT and BEADLE. This phenomenon takes place in such a way that the derived arrangement would be at no selective disadvantage in nature such as should result from egg inviability. In the male Sciara, synapsis is omitted and selective segregation occurs, so that a rearranged chromosome cannot be the instigator of aberrations leading to partial sterility. Data have been obtained which, contrary to previous genetic observations on other species of the genus Sciara, show that crossing over occurs in the X chromosome of *Sciara impatiens* in nearly 100 per cent of the maturing eggs.

MATERIAL AND METHODS

The stock of *Sciara impatiens* used for this study was established from flies collected in two greenhouses in the vicinity of St. Louis, Missouri, in May 1943, and has been maintained in the laboratory to date, using the usual culture methods for Sciara as outlined by SMITH-STOCKING (1936). Aceto-orcein smears of the salivary gland chromosomes showed that two of the most widely distributed single-inversion differences were present, one in the C chromosome and one in the X chromosome. Chromosomes A and B were homozygous for the standard gene arrangements. By the use of salivary gland smears to determine the presence of inversion heterozygosity in a given fraternity or sorority, it was possible to eliminate the C chromosome inversion from the stock. This was done by discarding all vials of flies in which salivary smears revealed the presence of the C chromosome inversion and selecting, for the perpetuation of the stock, sibs of individuals known to be homozygous for the standard C chromosome gene arrangement. Thus the only gene rearrangement present

in the stock when finally derived was the X chromosome inversion. This inverted chromosome (gene arrangement X-1, CARSON 1944) is of particular interest in that it involves almost the entire length of the chromosome, is to one side of the centromere (paracentric),[1] and is cytologically terminal in the salivary gland chromosome preparations (see fig. 4).

In *Sciara impatiens*, male- and female-producing females occur in approximately a 1:1 ratio. According to the scheme of METZ (1938) for this type of sex determination in Sciara, male-producing females are XX and female-producing females are X′X in sex chromosome constitution. In carrying out the selection process described above, it was noted that the single salivary gland X chromosome of males was invariably of the standard or uninverted gene arrangement. Conversely, X-chromosome configurations in any given sorority of females were either standard X/standard X or standard X/gene arrangement X-1. Thus it is obvious that the X′ ("X-prime") factor or group of factors is exclusively associated with the homologue carrying the X-1 inversion, the length of which prevents effective recombination of the X′ factor with the standard arrangement. This situation results in a balanced stock in which the female-producing females (X′X) are always heterozygous for the X-1 inversion, the male-producing females (XX) homozygous for the standard arrangement, and in which males always carry a single standard X chromosome. This stock was used exclusively in the following work.

The eggs were obtained and prepared for study in the following manner. Females were isolated soon after they emerged from the pupa, mated, and placed individually into vials. After a lapse of approximately 36 hours at 25°C, each female was etherized lightly and placed on its side on an agar block resting on a piece of moist filter paper in a petri dish. Oviposition was induced by crushing the thorax slightly with a blunt needle bent at an obtuse angle. Under these conditions, a properly-matured female will lay its eggs in a remarkably regular fashion, at the rate of five to eight a minute, and will continue until all its eggs (approximately 150) have been laid. The usual procedure was to isolate three or four groups of eggs at five-minute intervals; in doing this, the female, rather than the eggs, was moved to another position on the agar block, so as to avoid touching the eggs. These isolated groups of eggs were allowed to develop in the covered dish and then fixed after the desired length of time in 1:1:1 Carnoy's fluid. It was found necessary to rupture the chorion lightly with a finely-ground steel needle to ensure quick penetration of the fixative. The remainder of the eggs were placed in an ordinary culture vial and allowed to develop so that the sex of the progeny could be determined. Contrary to the observations of DUBOIS (1933) on *Sciara coprophila*, not a single instance of an unfertilized egg due to too hurried oviposition was found in more than 2500 timed eggs obtained in this way.

The eggs were stained *in toto* by the Feulgen technique, using the modifica-

[1] As will be shown later, the occurrence of bridges and fragments in the eggs of females heterozygous for this inversion precludes the possibility that the centromere is included in it (pericentric).

tions suggested by DE TOMASI (1936) and mounted entire, following the general procedure outlined by SCHMUCK and METZ (1932). Handling of the eggs was greatly simplified by pipetting them before staining into ant-cocoon "bags" in 70 per cent alcohol, as suggested by COOPER and RIS (1943). All drawings, with the exception of figures 1 and 4, were made with the aid of a camera lucida.

COMPARATIVE VIABILITY OF EGGS OF MALE-PRODUCING AND FEMALE-PRODUCING FEMALES

In order to determine if any egg inviability, as determined by hatchability, was evident in the eggs of females heterozygous for the X-1 gene arrangement

TABLE 1

Hatchability of eggs of females heterozygous (female-producers) and homozygous (male-producers) for X chromosome inversion X-1

FEMALE NO.	NO. OF EGGS LAID	NO. OF EGGS HATCHING TO LARVAE	% OF EGGS HATCHING TO LARVAE
Female-producers (X'X)			
1	37	30	81.1
2	127	125	98.4
3	139	135	97.1
4	149	146	98.0
5*	49	48	98.0
10*	113	108	95.6
12*	109	105	96.3
14	172	171	99.4
15*	104	99	95.2
Total	999	967	96.8
Male-producers (XX)			
6	128	125	97.7
7	156	152	97.4
8	210	196	93.3
9	105	103	98.1
11	139	125	89.9
13	182	174	95.6
Total	920	875	95.1

* Produced one or two "exceptional" males each.

(that is, female-producing females), a series of egg counts was carried out. In this experiment, the male-producing females, being homozygous for the standard X-chromosome gene arrangement, served as controls. Stock females were isolated and placed individually with two stock males each in creamers half-filled with a strained, smooth lamp-black-agar mixture. Most of the females laid eggs in scattered groups; if the eggs had been laid in large heaps, the vial was discarded in order to avoid the necessity of touching the eggs during the counting process.

The results of these counts are given in table 1. The total number of eggs laid by each female is recorded in the second column, and in the third the number of hatched larvae observed in each creamer. Comparison of the percentages of larvae hatching from the eggs of the two types of females shows that there is no reduced hatchability in the eggs of the female-producers (that is, those carrying the inverted X′ chromosome). In fact, the percentage of hatching larvae is slightly lower in the data for the male-producers (95.1 per cent as compared to 96.8 per cent for the eggs of the female-producers).

As male- and female-producing females cannot be distinguished morphologically, it was necessary to carry the hatched larvae through to adults in order that the type of each female whose eggs were counted could be determined. As there was considerable crowding and dessication in the creamers, the percentage of hatched larvae which grew and produced emerged adults is not reliable, but it was surprisingly high (71.0 per cent).

CYTOLOGICAL OBSERVATIONS

The meiotic divisions and the first cleavage mitosis in the eggs of male-producing females

As indicated previously, females of this type are homozygous for the standard gene arrangements, and their eggs thus serve as the normal controls for the eggs of the female-producing females, in which an inversion of the X′ chromosome is invariably present. Dicentric bridges and acentric fragments have not been observed in the meiotic divisions in the eggs of male-producing females, and the course of maturation after laying consistently follows the pattern shown diagrammatically in figure 1. In this figure, the size of the nuclear figures relative to the size of the egg as a whole has been somewhat enlarged for diagrammatic purposes. The average size of fixed eggs is 189μ in length and 98μ at the greatest diameter. The times given for the various stages after laying are averages, and considerable variation is encountered.

Studies of dividing brain-cell nuclei of females (CARSON 1944) have shown that the normal chromosome complement in this species consists of three pairs of rod-shaped chromosomes and one pair of V-shaped chromosomes, a total of eight. The three pairs of rods are all essentially equal in length; one of these is the X-chromosome pair, and the other two pairs correspond to salivary gland autosome pairs B and C. The V-shaped pair, which has arms of equal length, corresponds to salivary gland autosome pair A.

As in many other species of Sciara, the cells of the germ line, and consequently the maturing eggs and sperm of *Sciara impatiens*, usually possess, in addition to the normal chromosome complement, at least one large, peculiar, heteropycnotic chromosome termed by METZ (1938) the "limited" chromosome. It should be emphasized that these chromosomes are eliminated from all potential somatic cells during early cleavage and are thus "limited" in the sense that they are found in the cells of the germ line only. Their function is unknown, but as their number is variable and as they are lacking in some species, they have been thought to be inert and possibly vestigial in nature.

In a mature ovarian egg, or one fixed at or slightly after laying, the egg nucleus is found to be in the first meiotic division, arrested at early anaphase (fig. 5).[2] There are four or five dividing elements present on the spindle; when there are four (as in figure 5), these represent the three autosome tetrads (already divided to form anaphase dyads) and the X-chromosome tetrad. The fifth element which may be present is the dyad "limited" chromosome (for example, L, fig. 6). This chromosome passes undivided at random to either pole at the first division. In such an anaphase as that shown in figure 5 (one minute after laying), the dividing dyads of the A-chromosome pair are the only elements, other than the "limited chromosome," that can be identified with certainty; the arms of the V's which it forms are noticeably thicker, and the chromatids in them often diverge at the ends (as in A, figure 6).

If the egg has been fertilized, anaphase (fig. 7; no limited chromosome) is resumed at once, and telophase of the first meiotic division is reached approximately 30–40 minutes after laying at 21 degrees C (fig. 1A). The sperm nucleus, which has entered through the micropyle at the anterior end of the egg, lies in the cytoplasm at this end, where it is easily identified as a slightly twisted, Feulgen-positive streak.[3] The chromosomes of the egg nucleus move apart in a normal fashion, with the axis of the spindle directed toward the surface of the egg. The outer of the two chromosome groups, which is destined to be the progenitor of polar nuclei only, is designated as 1 and the inner group as 2 (fig. 1A, 7). The "limited" chromosome, if present, passes undivided to either pole; no egg has been observed in which more than one of these is present.

Following telophase of the first meiotic division a reorientation of the condensed chromosomes of nuclei 1 and 2 occurs, and at about 50 to 60 minutes after laying, the metaphase of the second meiotic division is reached (2, fig. 1B). The axis of the spindle which arises in connection with nucleus 2 is identical with that of the first division, but the polar nucleus (1) usually divides parallel, or nearly so, to the long axis of the egg. Anaphase and telophase of the second division follow (fig. 1C, 11), the nuclei dividing synchronously. In figure 11, the "limited" chromosome (L), which passed bodily into nucleus 1 at the first division, can be seen to have divided equasionally at the second division; thus 1a and 1b both contain a "limited" chromosome, whereas the definitive or oötid nucleus (2b) and nucleus 2a have none.

At approximately 75 minutes, the resulting four nuclei, 2b (oötid), 2a, 1b, and 1a are arranged as shown in figure 1D. Nucleus 2b immediately sinks deep into the center of the egg and approaches the sperm nucleus, which by this time has become rounded and, like the oötid nucleus, stains less intensely. The three polar nuclei, now also considerably paler, lie close to the surface and begin to move toward the posterior end of the egg; 2a and 1b remain in close apposition.

At 85 to 90 minutes, the egg and sperm pronuclei have formed a synkaryon

[2] In this figure, and in other similar ones, the line to the right of the figure represents the edge of the egg, and the anterior end of the egg is toward the top of the page (*cf.* fig. 1).

[3] No egg containing more than one sperm nucleus was encountered.

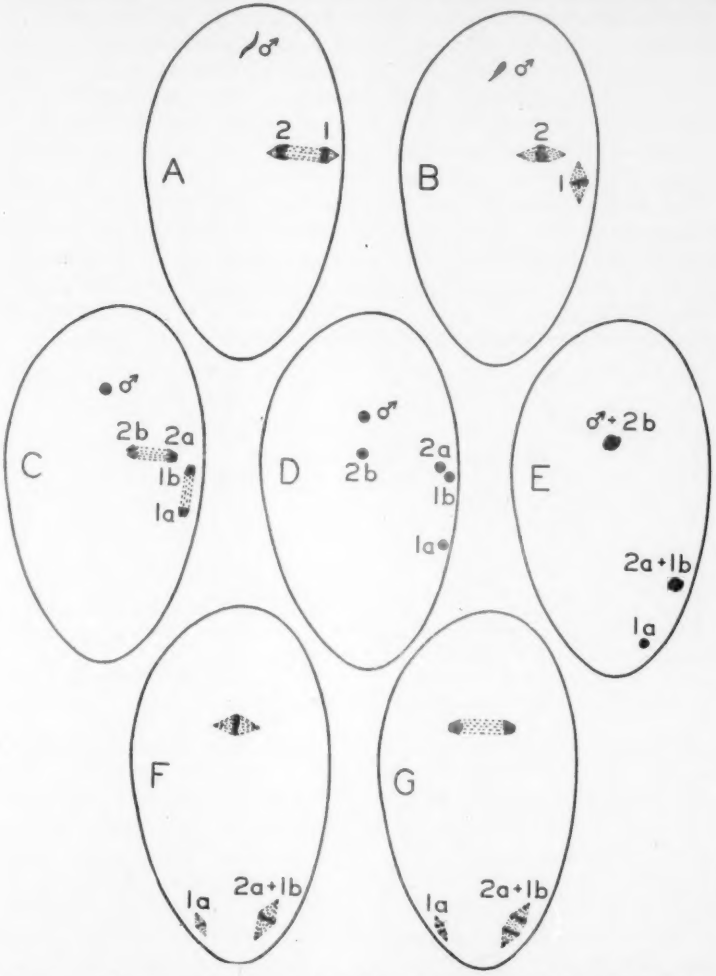


FIGURE 1.—Diagrammatic representation of the cytological events in the egg of *Sciara impatiens* from 30 minutes (A) until 120 minutes (G) after laying. The numbered nuclei (1a, 1b, 2a, 2b) are the products of the two meiotic divisions of the egg nucleus; 2b is the definitive or oötid nucleus. ♂ = sperm. For details, see text.

A. Telophase of first division, 30–40 min. Nucleus 1 will divide to form polar nuclei 1a and 1b.

B. Metaphase of second division, 50–60 minutes.

C. Anaphase of second division, 70–75 minutes. 2b is oötid nucleus; the others are polar nuclei.

D. Oötid stage, 75 minutes. 2b is approaching the sperm pronucleus.

E. Synkaryon formation, 85–90 minutes. Polar nuclei 2a and 1b have also formed a fusion nucleus.

F. Metaphase of first cleavage, 110–115 minutes. Migration of polar nculei to posterior end is complete.

G. Telophase of first cleavage, 120 minutes. Polar nuclei in anaphase.

toward the anterior end, deep in the center of the egg. Nuclei 2a and 1b have also formed a fusion nucleus, and along with 1a, continue to move rapidly toward the posterior end (fig. 1E). A fairly long prophase ensues, during which the polar nuclei come to lie at or near the extreme posterior end of the egg, and by 110–115 minutes, metaphase of the zygote nucleus has been reached (fig. 1F). A polar view of the zygote metaphase (fig. 2) shows the chromosome number that would be expected on the basis of studies on other species of Sciara (METZ 1938). In addition to the very large and heteropycnotic "limited" chromosome (L, fig. 2), which in this case may be either paternal or maternal in origin, there are nine chromosomes present. The "extra" chromosome above the diploid number is apparently one of the two sister X chromosomes transmitted by the sperm. The pair of V-shaped A chromosomes is again the only euchromosome pair that can be recognized.

FIGURE 2.—Metaphase of zygote nucleus in an egg of a female-producing female (*cf.* fig. 1F). L = "limited" chromosome. The V-shaped euchromosomes constitute autosome pair A; the "extra" rod is one of the two sister X chromosomes contributed by the sperm. 2100×.

FIGURE 3.—Metaphase of the dividing diploid polar nucleus (2a+1b) in the egg of a male-producing female (*cf.* Fig. 1F). L = "limited" chromosome. 2100×.

Although synchrony of all nuclei in the egg has been maintained up to this point, the polar nuclei are now somewhat out of phase with the zygote nucleus, usually lagging somewhat behind. Loss of synchrony appears to begin as soon as the polar nuclei have entered the "pole plasm" at the posterior end of the egg. Following their migration into the pole plasm, both polar nuclei (2a+1b is diploid; 1a is haploid) divide (fig. 1G). The axes of the polar body spindles are almost invariably longitudinal or somewhat oblique. The expected chromosome complement is found in the diploid polar body. Figure 3 shows a metaphase plate of this dividing diploid polar body (*cf.* fig. 1F; 2a+1b); eight chromosomes, in addition to the one larger "limited" chromosome (L) are present. Telophase of the first cleavage (fig. 1G) is reached at approximately 120 minutes after laying; the axis of its spindle is always transverse.

The meiotic divisions and the first cleavage mitosis in the eggs of female-producing females

It will be recalled that the female-producing females in this stock of *Sciara impatiens* differ from the male-producers in that one of the X chromosomes, the X′, always carries an inversion which involves practically the entire length

of the chromosome, whereas its homologue, the X, always has the standard gene arrangement. Although the early meiotic prophase has not been examined, the synaptic configuration formed by these two chromosomes in the salivary gland cell nuclei has been described and figured (CARSON 1944, fig. 50).

Figure 4 shows in diagrammatic fashion the arrangement of the standard X chromosome, the inverted X′ and the configuration formed when these two chromosomes synapse. The standard X chromosome (fig. 4, upper) includes regions 78 to 100. Salivary gland chromosome end 2 terminates in a heterochromatic mass, designated as 100d, which is known to represent the position of the centromere (see Discussion). The inverted X′ chromosome (gene arrangement X-1) differs in that practically the entire length of the chromosome to the left of 100d (the centromere region) is inverted, so that region 78, which was formerly in a terminal position at the distal end, is now brought into a

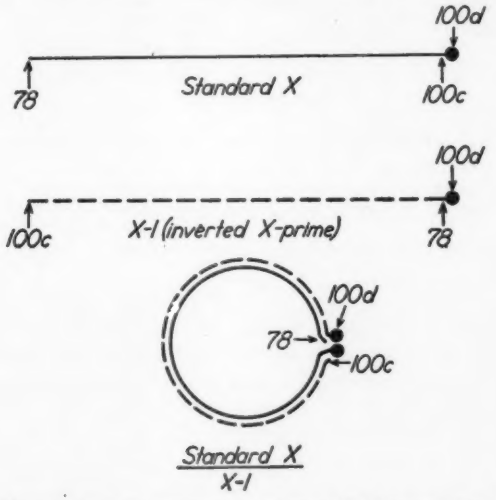


FIGURE 4.—Diagrams, based on studies of the salivary gland chromosomes, showing the gene arrangement of the standard X chromosome (upper), gene arrangement X-1 (middle), which differs from the standard X by a long terminal inversion, and (below) the configuration formed at synapsis between standard X and X-1. 100d is a heterochromatic mass which represents the position of the centromere. For details, see text.

position adjacent to 100d. Region 100c forms the distal end of the X-1 arrangement. Pairing is intimate and results in a typical terminal inversion loop (standard X/X-1, fig. 4), although in the salivary gland cell nuclei the proximal (or centromere) regions occasionally fail to synapse. Such a loop configuration is presumably formed at meiosis, and it is clear that any crossing over that occurs must of necessity be within the confines of the inversion. A single chiasma at any point would result in the formation of an anaphase bridge and fragment at the first meiotic division.

The general sequence of events after laying, described above for the male-producing females and figured diagrammatically in figure 1, is followed by the maturing eggs of the female-producing females. In the mature ovarian egg, an arrested anaphase (fig. 6) similar to that of the male-producers is found. All pairs of anaphase dyads can be seen to be "clearing" normally with the exception of one, presumably the X, in which the homologous centromeres are tied together by an anaphase bridge formed following chromatid exchange within the inversion. The acentric fragment has not yet moved away from the dividing chromosome, although in figure 6 the non-crossover chromatid to the

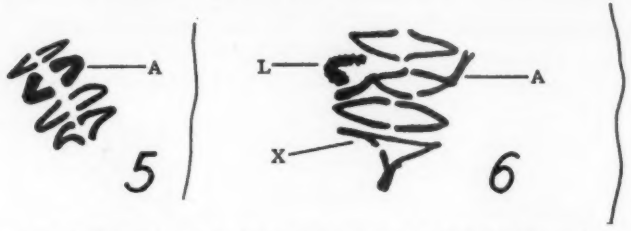


FIGURE 5.—Early anaphase of first division in an egg of a male-producing female, one minute after laying. A = A chromosome. The separating dyads at the top of the figure lay directly behind the A chromosome dyads; they were drawn above the latter for clarity. 2100×.

FIGURE 6.—Early anaphase of first division in a mature ovarian egg of a female-producing female. The X chromosome shows a dicentric bridge and a fragment which has not yet moved away from the non-crossover chromatid to the right. 2100×.

left can be seen to be clear of it. The A chromosome can easily be identified; figure 6 also shows an undivided "limited" chromosome moving into the inner nucleus (nucleus 2; *cf.* fig. 1A). Just before anaphase movement begins, the chromosomes become considerably shorter and thicker, as shown in figure 5 which is from an egg fixed one minute after laying (*cf.* fig. 6, which represents the nuclear figure of a mature ovarian egg).

Anaphase and telophase of the first division and metaphase of the second division in the eggs of female-producing females are especially interesting. Approximately 300 eggs in these stages were studied, and with a few possible exceptions, all showed clear dicentric bridges and acentric fragments. Because of the feasibility of rolling the egg into a favorable position by moving the cover-slip, it is ordinarily possible to analyze each figure with a high degree of accuracy. On the other hand, in an equal number of eggs of male-producing females, no such aberrations have been found, and the anaphase (fig. 7; no "limited" chromosome) and telophase of the first division are uniformly clear. Data on the frequency of bridges and fragments and on the various types that may occur are being gathered and will be presented elsewhere. The observations presented in this paper concern only those figures with a single bridge and single fragment.

Figure 9 shows a late telophase of the first division in an egg of a female-producing female. The "limited" chromosome (L) has moved into nucleus 1,

and three autosome dyads may be seen in each nucleus; again autosome A may be recognized by the divergence of the chromatids in its arms. As the telophase is reached, the chromatids of each dyad, which form an acute angle in anaphase (fig. 7), have now swung apart until they form a nearly straight line, parallel to the axis of the spindle. The centromeres are located at the middle of the very wide-open V's. The configuration formed by the X chromosome is interpreted as a dicentric bridge chromatid to which the non-crossover chromatids are still attached at the centromeres. In figure 9, the position of the inner

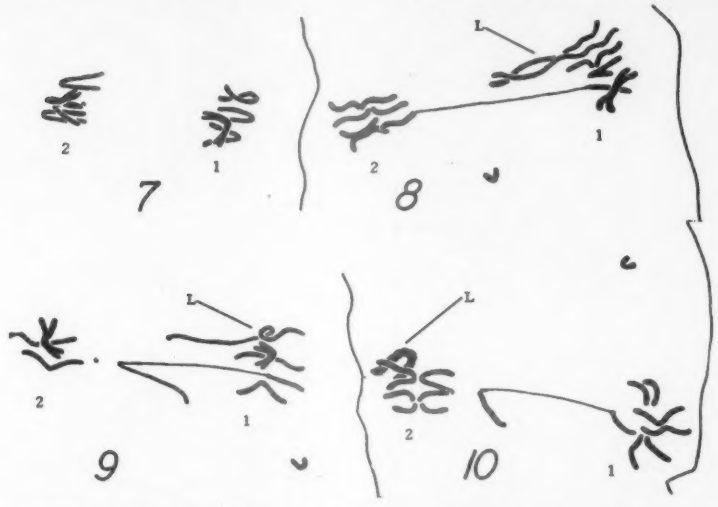
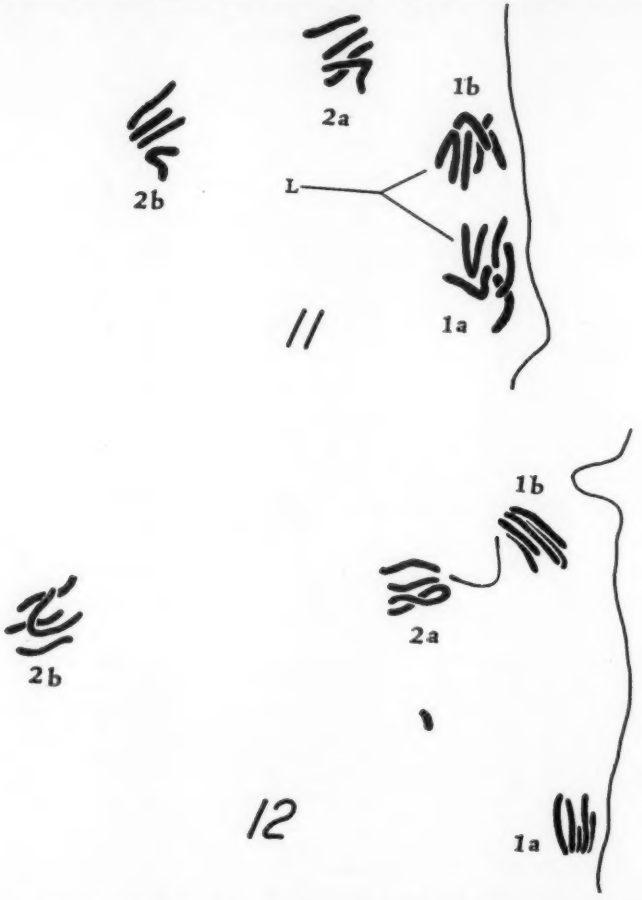


FIGURE 7.—Anaphase of first division in an egg of a male-producing female, 40–45 minutes after laying. 1 and 2 designate outer and inner nuclei respectively (*cf.* fig. 1A). 1531×.

FIGURES 8, 9, AND 10.—Metaphase of second division (fig. 8; 40–45 minutes), late telophase of first division (fig. 9; 28–35 minutes) and very early anaphase of second division (fig. 10; 40–45 minutes) in eggs of female-producing females. All show dicentrics (with non-crossover chromatids still attached at the centromeres) and fragments. 1 and 2 designate outer and inner nuclei respectively (*cf.* fig. 1B). L = "limited" chromosome. For details, see text. 1531×.

centromere (to the left) of the dicentric is at the apex of the V formed by the dicentric and the non-crossover chromatid. Although at this stage the dicentric is always somewhat attenuated, its centromeres are rarely, if ever, parallel with the centromeres of the other dyads. Thus the whole dividing chromosome tends to be somewhat displaced toward the center of the figure (see fig. 8, 9, and especially 10). The acentric fragment, such as that to the lower right in figure 9, has moved away from its original position close to the bridge; such behavior is universally encountered. In all the figures studied, the fragment appears to be of constant size and, at this stage, usually V-shaped. Figures 8 and 10 represent metaphases of the second division; figure 10 might be considered an early anaphase of the second division, in that the chromatids

of the chromosomes other than the X have already disjoined at the centromere. The earlier orientation of the chromatids in a poleward direction from the equator tends to be retained, as is seen clearly in nucleus 2 of figure 10. In this

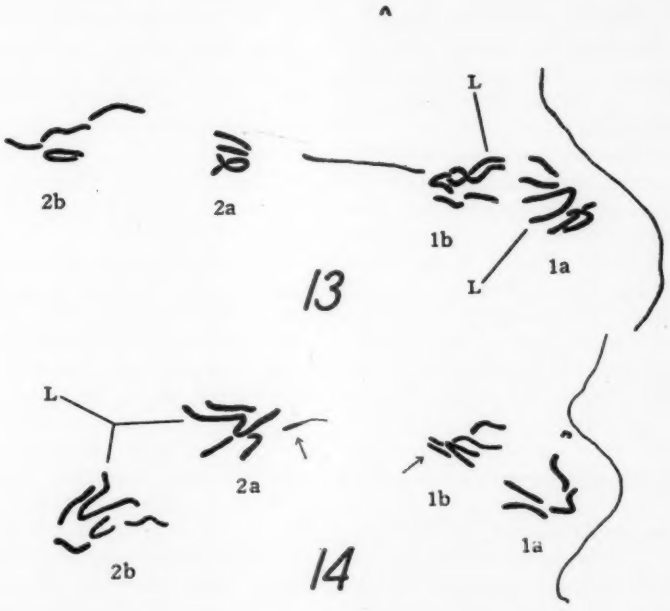


FIGURES 11 AND 12.—Late anaphase of second division in egg of male-producing (fig. 11, 85–90 minutes) and female-producing (fig. 12, 70–75 minutes) females. 2b is the oötid nucleus; the others are polar nuclei (*cf.* fig. 1C). In figure 12, the dicentric chromatid lies between 2a and 1b; 2b has four chromosomes; 2a only three. The "limited" chromosome has presumably been divided equasionally between 1a and 1b, but cannot be identified. 2100×.

figure, the centromeres of the X chromosome lie at the points where the thicker non-crossover chromatids join the ends of the dicentric, forming with it an acute angle (left-hand one) and an obtuse angle (right). The non-crossover

chromatid to the left appears to be just disjoining from the left end of the dicentric.

In order that the inner nucleus of the eventual four, which is destined to be the oötid nucleus (2b), might obtain a complete haploid set of chromosomes, it would be necessary for the non-crossover chromatid at the inner end of the bridge to move from its position in figures 8, 9, or 10 all the way to the left, so as to pass to the same pole, for example, as the three left-most autosome chromatids in figure 10. Study of second division anaphases shows that this is, indeed, what happens (fig. 12, 13, 14; *cf.* fig. 11, a comparable stage in a male-



FIGURES 13 AND 14.—Anaphases of second division in eggs of female-producing females, approximately 60 minutes after laying. In each, four euchromosomes can be seen in 2b and 1a, whereas 2a and 1b contain only 3. (*cf.* fig. 1C). "Limited" chromosomes (L) are present and have segregated at random. Figure 14 shows a broken dicentric; its two parts are indicated by arrows. For details, see text. 2100×.

producing female). In figure 12, four chromatids, three rods and one V may be seen in the oötid nucleus (2b), whereas 2a contains only three. The dicentric chromatid, often bent at this time, may be seen between the two inner nuclei (2a and 1b). That the other non-crossover chromatid moves into the terminal polar nucleus (1a) may be seen in figures 13 and 14, which show slightly earlier

stages of anaphase II. In figure 13, nucleus 2b has four chromosomes, one of which is a V (the A chromosome); 2a has three. The unbroken dicentric chromatid extends between 2a and 1b, which has only three euchromosomes. There are four euchromosomes in the outer terminal nucleus (1a), and both 1a and 1b contain an additional "limited" chromosome (L) which has been divided equasionally between them at the second division (*cf.* fig. 11). The fragment in figure 13 may be seen above the bridge, far away from its original position. These observations have been confirmed in approximately 20 exceptionally favorable figures at this stage from a number of females, and no exceptions to the above behavior, in which the two non-crossover chromatids move into the terminal nuclei, have been encountered.

A number of cases in which the bridge has been broken during late anaphase of the first division have been found. Figure 14 shows a second division anaphase in an egg in which such an event occurred. Again, nucleus 2b possesses a full complement of chromosomes, together with an additional "limited" chromosome, whereas nucleus 2a contains three entire euchromosomes, a "limited" chromosome, and a portion of the broken bridge (at arrow); 1b also has three euchromosomes and the other portion of the broken bridge (arrow), while 1a as usual, has a full haploid complement. In this figure the fragment is close to the outer edge of the egg, slightly above nucleus 1a.

The subsequent events are comparable to those in the eggs of the male-producing females. Nucleus 2b migrates deep into the center of the egg and forms a normal synkaryon with the sperm pronucleus; the former may or may not transmit a limited chromosome. Nuclei 2a and 1b unite to form a diploid polar nucleus in which the dicentric or its broken parts are presumably included, although the fate of the dicentric has not been traced. The division of the diploid polar nucleus is difficult to study in the whole eggs, since the axis of the spindle is almost universally longitudinal, so that the egg usually cannot be rolled into a position which would permit study of the metaphase group.

When the migration of 2a+1b and 1a toward the pole plasm takes place, the acentric fragment, which up to this time has been behaving at random, also enters into this migration and is usually found lying free in the cytoplasm at the posterior end near the dividing polar body nuclei. Immediately following the telophase of the second division, all the nuclei become very pale, and during this time the fragment is difficult to find, since it remains in synchrony with the nuclei, forming a minute, pale vesicle. When the polar nuclei come into prophase, however, the fragment is again easily detected as an elongated prophasic thread lying free in the cytoplasm. It again reaches a maximum of condensation at the time when metaphase of the polar nuclei is reached, even though it may be quite far removed from either polar nucleus.

The times given in the description of the events following laying in the eggs of the male-producing females are essentially the same for the eggs of the female-producers. It is evident, therefore, that the formation of anaphase bridges at the first division causes no delay in the completion of maturation. Delayed eggs from both male- and female-producing females are occasionally found;

these are cytologically normal in every way, and there is no reason to believe that such eggs would be inviable.

DISCUSSION

In recent years, it has become clear that the inversion of a chromosome segment is one of the most common types of natural chromosomal aberrations, occurring widely in animals and plants (DARLINGTON 1937; DOBZHANSKY 1941). Since the salivary gland chromosome method is exceptionally well adapted for studies of this kind, analysis of the occurrence of inversions in natural populations in the Diptera (for example, Drosophila, Sciara, and Chironomus) has been more detailed and exact and has proceeded further than in other organisms. Although these dipteran genera show numerous inversions, there is no reason to believe that this apparent high frequency is exceptional.

MULLER (1940) has classified inversions into two types, depending on whether the centromere is included in the inversion (pericentric) or whether the inversion is to one side of it (paracentric). This distinction is of considerable importance because direct observation of the cytological results of crossing over within the inversion permit relatively easy detection of the presence of paracentric inversions, whereas detection of pericentric inversions in this way is not possible. A single crossover, as well as most types of doubles, within a paracentric inversion leads regularly to the formation of dicentric bridges and fragments at the first meiotic division. In organisms in which it is impractical to demonstrate the presence of inversions genetically or by a study of synapsed chromosomes, these meiotic bridges and fragments have been widely used as an indication of inversion heterozygosity. On the other hand, crossing over within a pericentric inversion leads to the formation of duplication-deficiency chromatids which, under ordinary circumstances, are unrecognizable cytologically. For this reason, the detection of paracentric inversions is selectively favored, and as a result, little information is available concerning the frequency and distribution of pericentric inversions.

The distinction between paracentric and pericentric inversions has further important implications. The anaphase bridge and fragment following crossing over within a paracentric inversion results in the inclusion of an aneuploid complement of chromosomes in two of the four theoretically possible gamete nuclei. Likewise, following crossing over within a pericentric inversion, half of the resulting nuclei would also be aneuploid, due to the segregation of duplication-deficiency chromatids. Thus, superficially, crossing over within both types of inversions would appear to have essentially the same result: half of the gametes produced would be aneuploid and, since these would presumably be inviable, individuals heterozygous for the inversion in nature would be subject to adverse selection. Naturally-occurring pericentric inversions, however, appear to be very rare. This is true at least for Drosophila (MULLER 1940; DOBZHANSKY 1941) in which the only known case is that described by MILLER (1939) for *D. algonquin*. No certain cases of pericentric inversions have been found in Sciara (CARSON 1944; MCCARTHY 1945). The

question thus arises as to how the paracentric inversion, which theoretically should be responsible for a certain amount of sterility, is nonetheless able to become firmly established in natural populations along with the uninverted arrangements, and, further, why the same is not true for pericentric inversions.

An ingenious hypothesis to answer this question has been provided by STURTEVANT and BEADLE (1936). Based on genetic evidence derived from an extensive study of the results of crossing over in X-chromosome inversion heterozygotes in *Drosophila melanogaster*, these authors have suggested that, as the polar bodies come off in a straight line, directed radially from the definitive nucleus, the dicentric chromatid should remain between the two inner nuclei. Thus each terminal nucleus, the inner one of which is the oötid, would regularly receive a non-crossover chromatid. This would effectively "protect" the oötid nucleus from aneuploidy and explain the fact that no reduced viability is associated with the presence of heterozygous paracentric inversions.

DARLINGTON and LA COUR (1941) were able to confirm this scheme in the embryo sac of *Lilium testaceum* and of a cross between diploid and triploid forms of *Tulipa Gesneriana*. In these forms, first division bridges were observed between the middle nuclei of the four produced at the second division. They are thus excluded from the definitive micropylar nucleus, which is terminal in position.

In the present paper, cytological data are presented which are in full agreement with the hypothesis of STURTEVANT and BEADLE and the substantiating evidence of DARLINGTON and LA COUR. Since bridges and fragments are regularly formed in the eggs of females of *Sciara impatiens* heterozygous for the long X chromosome inversion studied, it is concluded that this inversion is paracentric. Furthermore, as the inversion is terminal and involves end 1 (region 78), it is clear that end 2 (region 100d) of the salivary gland X chromosome is the centromere end (see fig. 4). Of the five gene arrangements (one hypothetical) described for the X chromosome of this species (CARSON 1944), all are very long and involve in each case a major portion of the chromosome. None, however, involves region 100d, now known to be at the position of the centromere. Counts show that no egg inviability accompanies inversion heterozygosity and indicate that a mechanism for the relegation of a euploid complement of chromosomes to the oötid nucleus must also be operating in the present case.

Cytological observations of the meiotic divisions in the egg show that first division bridges and fragments are formed in nearly 100 per cent of the eggs of females heterozygous for the inversion. This large number is probably a reflection of the great length of the inversion, in that any chromatid exchange must of necessity be within the confines of the inversion (see fig. 4). This shows, furthermore, that exchange between the X chromosomes in *Sciara impatiens* is very frequent; it approaches 100 per cent, since no clear-cut cases of bridge-free first divisions have as yet been encountered. This fact is significant in that genetic studies have led METZ and SMITH (1931) and METZ and SCHMUCK

(1931) to believe that the rate of crossing over in the X chromosome of *S. coprophila* is very low. In this connection, it is interesting to note that in this stock of *Sciara impatiens* no instance of recombination between the X′ factor and the standard arrangement has been recovered. Such an event could be expected only after a two- or three-strand double exchange and, as the chance of such an occurrence would be slight, it is evident that this section of the germ plasm is effectively isolated.

The details of the fate of the dicentric chromatid during the first and second divisions in *S. impatiens* are such that a complete haploid set of chromosomes is assured to the oötid nucleus. As anaphase I proceeds, the dicentric becomes considerably attenuated and, despite the obvious stress upon it, usually does not break. This resistance to breakage by the bridge results in a lagging of the whole chromosome to the effect that the non-crossover chromatids, which are attached to the dicentric at the centromeres, are held toward the center of the figure. Although their disjunction from the dicentric is somewhat delayed, both non-crossover chromatids overcome this "handicap" and move regularly into the terminal nuclei. Furthermore, even if the dicentric is broken at anaphase or telophase of the first division, this directed segregation of the non-crossover chromatids into the terminal nuclei occurs (see fig. 14).

Such behavior indicates an important role for the centromere in the movement of these chromatids. Reference to figures 8, 9, and 10 will show that the centromeres of the X element are outside of the general spindle influences of the second divisions, although it cannot be excluded that the shape of the spindles may be distorted to accommodate them. Descriptively speaking, however, the non-crossover chromatids must move past the migrating chromatids of the inner nuclei (2a and 1b) in the opposite direction to the movement of the latter in order to reach their final destination in the terminal nuclei. This movement gives the impression of being due, initially at least, to a repulsion between the centromere of the dicentric and that of the migrating non-crossover chromatid. The dicentric itself, however, does not move. This is indicated by the fact that it does not become bent until later, when the inner nuclei (2a and 1b) move toward one another (fig. 12). This fact would suggest that the repulsion, if it exists, is not mutual, and movement is confined to the non-crossover chromatid. It will also be recalled that observations of the mode of disjunction of ordinary dyads in the second division also indicate that the centromere itself plays a dominant role in the initial separation of the chromatids.

Thus it appears that the genetic and cytological evidence for the selective elimination of the meiotic aberrations caused by exchange within paracentric inversion heterozygotes in the dipteran egg is complete. Since there is no opportunity for exchange between homologues during spermatogenesis in either Drosophila or Sciara, such inverted arrangements appear to be at no selective disadvantage in nature. Although the scheme given above presumably operates rather generally in oögenesis in animals and in megasporogenesis in higher plants, the "protection" of the inverted arrangement would not be

achieved in the formation of male gametes in which synapsis, crossing over, and random segregation occur. In such an organism, half of the male gametes would usually be inviable after crossing over, resulting in selective disadvantage. This disadvantage should be realized particularly with respect to long inversions, since the chance of a crossover within the inversion, under most conditions, is directly proportional to its length. Thus the conditions in Drosophila and Sciara are especially favorable for the establishment of long paracentric inversions, since no mechanism operates against their perpetuation. Such conditions prevail in a number of organisms, such, for example, as the Hymenoptera, where male haploidy and abortive spermatogenesis should "protect" long inversions in the male. On the other hand, organisms with conventional male meiosis should tend to eliminate long paracentric inversions and retain only the shorter ones. Data on the length of inversions in organisms other than the Diptera are few, but certain observations, such as those of SWANSON (1940, 1941) on Tradescantia and GEITLER (1938) on Paris indicate that inversions in such forms are short and numerous.

The absence of any special mechanism as the above for the elimination of duplication-deficiency gametes which result from crossing over within pericentric inversions appears to be largely responsible for the general infrequence of this type of inversion in natural populations. The establishment of pericentric inversions, as MULLER (1940) and DOBZHANSKY (1941) have pointed out, may be favored under certain breeding and population conditions. Further analysis of their dynamics, however, depends upon the development of some generally applicable method for detecting their presence.

SUMMARY

Studies were made on a balanced stock of *Sciara impatiens*, in which half of the females (the female-producers) are always heterozygous for a very long paracentric inversion of the X chromosome, and the other half (the male-producers) are always homozygous for gene arrangement. Egg counts show that no inviability is associated with inversion heterozygosity.

Cytological study of more than 2500 eggs fixed at varying times after laying reveals the presence of practically 100 per cent first division dicentric and acentric chromatids (resulting from crossing over within the inversion) in the eggs of females heterozygous for the inversion. Eggs of homozygous females show none. This high percentage is attributed to the great length of the inversion and provides evidence that a hitherto unsuspected high frequency of chromatid exchange occurs between the X chromosomes of Sciara.

At the second division, the dicentric chromatid, which usually does not break, remains between the two inner nuclei of the four formed at meiosis. This results in a directed segregation of the non-crossover chromatids into the terminal nuclei, one of which is the functional, or oötid, nucleus. This nucleus, therefore, regularly receives a euploid complement of chromosomes.

These observations constitute a direct cytological corroboration of the hypothesis of STURTEVANT and BEADLE (1936) which was advanced to explain

the genetic results obtained from a study of crossing over within paracentric inversions in *Drosophila melanogaster*.

The importance of the selective elimination of dicentrics at meiosis in the female with respect to the establishment of paracentric inversions in nature is discussed, and it is emphasized that conditions in such organisms as Drosophila and Sciara where meiotic exchange is confined to the female especially favor the establishment of long paracentric inversions. On the other hand, the general rarity of pericentric inversions is attributed to the random segregation of duplication-deficiency chromatids produced by crossing over within such inversions; this results in an uncompensated selective disadvantage for the derived arrangement.

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SUMMARIES FROM FORTHCOMING PAPERS

WRIGHT, SEWALL, and TH. DOBZHANSKY. *Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by selection in certain natural populations of Drosophila pseudoobscura.*—Received October 3, 1945.—Artificial populations of *Drosophila pseudoobscura* were kept in cages constructed as a modification of the model devised by L'Héritier and Teissier.

All the flies used in the experiments reported in this paper are descendants of parents collected at Piñon Flats, Mount San Jacinto, California. Three types of third chromosomes, Standard, Chiricahua, and Arrowhead, are common in the population of that locality. Their relative frequencies undergo cyclic seasonal changes—namely, Standard increases and Chiricahua decreases in frequency during the summer; the opposite change takes place during spring; the relative frequencies remain constant during fall and winter.

Most of the experiments began with approximately equal numbers either of two or of three chromosome types. At 25°, with initial frequencies equal or with Chiricahua more frequent than Standard, there was a highly significant increase in frequency of Standard (where present) at the expense of either of the other types but especially of Chiricahua. On the other hand, there were no significant changes in mean frequency at 16.5°C. The rate of change at 25° showed a strong negative regression on frequency in all cases, and a probably significant regression of this sort was observed in one case (Arrowhead) at 16.5°. The data further indicate that all chromosome types are favored when sufficiently rare and opposed when sufficiently common.

The simplest hypothesis is that the heterozygotes are favored over both corresponding homozygotes and that there are no sex differences. The results in the experiments at 25° in which only Standard and Chiricahua were present can be adequately explained by postulating the relative selective values .70:1.00:.30 for ST/ST, ST/CH, and CH/CH, respectively. Equilibrium is indicated at 70 per cent Standard, 30 per cent Chiricahua.

However, there is no assurance that selection acts alike on males and females. The mathematical theory for unequal selective values in the sexes is presented. It is found that the results differ little except in extreme cases from those found with sex equality in selection. Thus the data can be fitted substantially as well by assuming selective values 1.00:1.00:.60 for females, .40:1.00:0 for males (for ST/ST, ST/CH, and CH/CH, respectively) as under the previous hypothesis in which both sexes are selected according to the averages of those figures.

It is possible that the selective values may vary with the changes in composition of the population. The mathematical theory of variable selection coefficients is discussed briefly. An extreme hypothesis of this sort (heterozygotes always intermediate while each homozygote decreases in selective value with increase in frequency) was found to fit the data reasonably well. The selective values arrived at were $(1.90-1.29q)$ for ST/ST, 1.00 for ST/CH, and $(.10+1.29q)$ for Ch/CH, where q is the frequency of Standard (.70 at equilibrium as before).

The mathematical theory of selection among multiple alleles is considered in connection with the cases in which three chromosome types were present. The selective values at 25°, indicated by the method of least squares, are .43 for ST/ST, .05 for AR/AR, .21 for CH/CH, 1.30 for ST/AR, 1.00 for ST/CH, and .71 for AR/CH. Equilibrium is indicated at 53 per cent Standard, 34 per cent Arrowhead, and 13 per cent Chiricahua. The hypothesis that all three heterozygotes are equal in selective value gives impossible results.

The experimental results demonstrate clearly that there may be selective differences between chromosome types derived from the same locality and that these may be of such a nature as to result in the indefinite persistence of several types in such a locality. The marked rise in frequency of Standard and decrease of Chiricahua in summer, observed to occur in nature in the locality from which the flies were collected, is analogous to the experimental reaction to high temperature, and it is tempting to compare the lack of change in artificial population at 16.5° with the constancy observed in the natural ones during fall and winter. The changes which occur in nature during spring (the reverse of those in summer), however, have not been reproduced in the population cages. Mass migration at this time from the population high in the mountains would explain the results qualitatively but is difficult to reconcile with the results of earlier experiments on the rate of dispersion of the flies in nature. It appears that there are important factors yet to be discovered.

SNELL, GEORGE D., *An analysis of translocations in the mouse.*—Received, October 4, 1945.—From a group of radiation-induced translocations in mice, six were selected for intensive study. These were: *T(5;8)a*, *T(1;?)c*, *T(2;?)d* *T-F*$_1$*111*, *T-F*$_1$*194*, and *T-R*$_1$*8*. These were tested for linkage against the genes *a*, *b*, *Ca*, *d*, *f*, *Fu*, *ln*, *p*, *s*, *sh-2*, *v*, *W*, *wa-1*, and *wa-2*, and in the case of *T(1;?)c* against *je*, *Re*, and *Sp* also. No linkages were found in the case of *T-F*$_1$*111*, *T-F*$_1$*194*, and *T-R*$_1$*8*, and these were therefore discarded. *T(5;8)a* was found to involve chromosomes 5 and 8, the break being about one unit from *a* and 19 units from *pa* on chromosome 5, and 21 units from *m* and 29 units from *b* on chromosome 8. *T(1;?)c* was found to involve chromosome 1, the break being very close to *c*, with crossing over somewhat reduced in the *c-p* segment on one side and the *c-sh-1* segment on the other. Semi-sterility in the case of *T(2;?)d* shows linkage with *d*.

By the use of linked genes it was possible to establish accurately the ratio of semi-sterile to normal from the mating semi-sterile×normal. The expected 1:1 ratio was closely approximated.

The fertility of the different translocation heterozygotes was tested by comparing the mean litter size from the mating semi-sterile×normal with that from comparable normal×normal matings. The values, expressed as percentages, ranged from 38.4 to 62.2 for females and from 41.3 to 68.9 for males. The differences from 50 per cent are unquestionably significant in the case of four of the translocations. In the case of *T(5;8)a*, the fertility percentage is about 50 in the female and 61 in the male, the results being consistent in three

entirely distinct crosses and unquestionably significant. Five of the other six translocations also show greater fertility in the male.

T(5;8)a and *T(1;?)c* are viable in the homozygous condition, though there is a suggestion of a reduced viability in the case of the latter. The other translocations were not tested.

A mating of *T(5;8)a* heterozygote×heterozygote, with appropriate marker genes, showed that *pa* and *b* lie on opposite arms and that non-disjunction may occur along both axes but is most common on the axis which does not carry the marker genes. Since centromeres must lie on opposite arms, it can be stated that if the centromere lies on the pallid end of the *pa-a* chromosome, it must lie on the brown end of the *b-m* chromosome, and vice versa.

A mating of *T(1;?)c* heterozygote×heterozygote with appropriate marker genes failed to produce either of the possible non-disjunction classes of young. The litter size was also unexpectedly small. Possible explanations for this are discussed.

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